On the Road to Translation for PTSD Treatment: Theoretical and Practical Considerations of the Use of Human Models of Conditioned Fear for Drug Development

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Abstract The use of quantitative, laboratory-based measures of threat in humans for proof-of-concept studies and target development for novel drug discovery has grown tremendously in the last 2 decades. In particular, in the field of posttraumatic stress disorder (PTSD), human models of fear conditioning have been critical in shaping our theoretical understanding of fear processes and importantly, validating findings from animal models of the neural substrates and signaling pathways required for these complex processes. Here, we will review the use of laboratory-based measures of fear processes in humans including cued and contextual conditioning, generalization, extinction, reconsolidation, and reinstatement to develop novel drug treatments for PTSD. We will primarily focus on recent advances in using behavioral and physiological measures of fear, discussing their sensitivity as biobehavioral markers of PTSD symptoms, their response to known and novel PTSD treatments, and in the case of d-cycloserine, how well these findings have translated to outcomes in clinical trials. We will highlight some gaps in the literature and needs for future research, discuss benefits and limitations of these outcome measures in designing proof-of-concept trials, and offer practical guidelines on design and interpretation when using these fear models for drug discovery.

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1   Introduction

1.1   Posttraumatic Stress Disorder Prevalence and Treatment Options

Posttraumatic stress disorder (PTSD) affects 7–8 % of the general US population and is higher in recently deployed combat veterans (up to 20 %) (Thomas et al. 2010). Mental disorders, in particular PTSD, are associated with higher rates of physical symptoms, chronic physical illness, and overall mortality (for review see Baker et al. 2009). Research shows that this increased liability of physical disease translates into greater non-mental health medical service utilization (e.g., O'Donnell et al. 2013), creating substantial burdens for the patients, families, and societal resources. Best evidence treatment for PTSD includes cognitive behavioral therapies, i.e., cognitive processing therapy (CPT) and prolonged exposure (PE), and psychotropic medications (Institute of Medicine 2014). Although cognitive behavioral approaches have proven efficacy for PTSD, non-response can be as high as 50 %, leaving unresponsive or partially responsive patients with PTSD reliant upon pharmacotherapy (Baker et al. 2009; Institute of Medicine 2014; Berger et al. 2009). As with many psychiatric disorders, the pharmacological tool kit for PTSD treatment is relatively small, predominantly selective serotonin or norepinephrine reuptake inhibitors (SSRI/SNRI) and adjunctive treatments such as prazosin, a sympatholytic drug with alpha-1 receptor blocking activity (Baker et al. 2009; Steckler and Risbrough 2012). These medications also have high non-response rates as well as side effects (Baker et al. 2009; Steckler and Risbrough 2012). There is an unquestionable need to advance development of new treatments for PTSD, with part of this effort lying in developing innovative approaches to drug development in clinical populations.

One of the difficulties of identifying biological mechanisms for PTSD, and thus in turn developing beneficial treatments, is the heterogeneous patient population and wide spectrum of potential symptoms. According to the DSM-5 (American Psychiatric Association 2013), PTSD now comprises 20 individual symptoms. These symptoms are grouped into four symptom clusters: persistent intrusive memories of the trauma, hyperarousal and reactivity, avoidance of stimuli related to the trauma event, and negative alterations in cognitions and mood. Thus, there is a wide range of symptoms that can be endorsed to comprise a PTSD diagnosis, with many possible patterns of symptom type and severity across these clusters (Galatzer-Levy and Bryant 2013). This heterogeneity suggests that several potential biological mechanisms could drive the development of PTSD. This multiplicity of potential biological mechanisms will induce substantial variance in how any given treatment will affect a patient's treatment response.

As such, the potential for numerous different underlying pathologies in patient groups makes identification of specific mechanisms across the population very difficult. One approach to this problem is to identify biological or behavioral phenotypes that are highly represented in the diagnostic class compared to specific symptoms so as to target a "core" biological pathway that is disrupted in most patients. This approach assumes that the heterogeneity is due to noise in the self-report measurements of symptoms and how they are experienced and/or articulated, but perhaps only a few biological mechanisms actually drive clinical dysfunction. The second potential approach is to identify phenotypes that are relevant to particular symptom classes that are most severe in a given individual. This approach assumes that certain discrete phenotypes may better classify dimensions of specific symptoms experienced by subpopulations within the diagnostic group as a whole, each with potentially differing biological mechanisms (Schmidt 2015).

Development of laboratory-based behavioral measures of disease-related processes is a critical component of the evolution of translational research (Bowers and Ressler 2015). These tasks can bridge complex clinical presentations with discrete biological mechanisms (Braff 2015; Gottesman and Gould 2003; Rasetti and Weinberger 2011; Risbrough 2010). This strategy is now endorsed by the Research Domain Criteria (RDoC) project by the National Institute for Mental Health (Cuthbert and Insel 2013). Similarly, industry and academia have now increasingly turned to biological and behavioral markers in initial proof-of-concept studies to identify efficacy across specific emotional and cognitive constructs of PTSD to guide future phase II clinical trial designs. Here, we will discuss the promise and pitfalls of commonly used laboratory-based measures of conditioned fear processes to support novel drug development for PTSD.

1.2 Considerations of Benefits and Limitations of Laboratory-Based Measures of Behavior for Drug Discovery

1.2.1 Benefits of Validated Behavioral Phenotypes to Complement Symptom Assessments

(1) Objective, quantifiable assessments of function compared to self-report.
(2) Often have well characterized biological mechanism(s) and neural circuit(s).
(3) Responses are predictably controlled by specific experimental parameters in keeping with their use as an operational measure of a defined construct (e.g., anxiety, fear, arousal).
(4) Observable behaviors enable cross-species translation to lower order organisms for direct mechanistic studies and drug development (Donaldson and Hen 2015).
(5) Compared to symptoms, laboratory-based measures are observable across healthy controls and clinical populations, supporting efforts to disentangle mechanisms that cause risk versus mechanisms related to symptom onset and severity. This point is particularly important for informing treatment approaches, e.g., prophylactic versus therapeutically.
(6) Unlike symptoms, behaviors can be measured in unaffected relatives to aid in identification of genetic risk factors [e.g., behavioral endophenotypes or intermediate phenotypes (Lenzenweger 2013)].
(7) Because they are typically based on continuous measures, they offer more statistical power than dichotomous diagnostic classes.
(8) Most importantly for drug discovery, they may probe a more specific conceptual target for pharmacotherapy indicated by preclinical studies (e.g., effective for enhancing fear extinction). This last point is the primary reason behavioral tests are being used more frequently, as they may offer a greater ability to translate drug effects that are based on specific circuit actions and behavioral effects in preclinical models.

1.2.2 Limitations

(1) Lack of specificity: It is often the case that some individuals with disrupted performance in a behavioral task may not show overt functional deficits or clinical presentation. For example, menstrual cycle phases are associated with reductions in fear extinction in healthy women (Glover et al. 2015; Milad et al. 2006).
(2) In the context of genetic studies, even relatively "simple" or discrete laboratory-based behaviors do not guarantee greater heritability or simpler genetic architecture than the disorder (Greenwood et al. 2007), as would be

hoped from an intermediate phenotype or endophenotype. For example, even a behavior as simple as the startle reflex may be modulated by a huge array of biological pathways (Zhang et al. 2011).

(3) Behaviors that initially seemed relatively simple in terms of core neural circuit, e.g., extinction requiring prefrontal cortex activation of inhibitory circuits in the amygdala, can have extensive modulatory circuits that may play a stronger role in how this phenotype is altered in a given disorder compared to the "core neural circuit" (Acheson et al. 2015c; Maren and Holmes 2015; Milad et al. 2013). Thus, using behavioral performance as a proxy for the function of a specific neural circuit or brain region is limited unless it is accompanied by other information such as functional imaging.

Here, we will review the state of the art in laboratory-based measures of fear response in assessing symptom state and response to treatment in healthy controls and PTSD patients within the fear learning domains. We will also offer some practical considerations for study design and interpretation pitfalls for future planning of drug efficacy using these measures.

2 Learned Fear Processes

One of the predominant features of PTSD symptoms is robust, uncontrollable memories of the traumatic event, i.e., re-experiencing. Secondly, external or internal cues that act as trauma reminders induce re-experiencing with flashbacks and dissociation at the most severe end of the spectrum, as well as strong emotional and physiological fear responses including intense anxiety and panic. Unsurprisingly, the disorder is associated with implicit and explicit strategies for cue avoidance, which can be disruptive to daily function and interfere with long-term recovery. Thus, PTSD may be caused at least in part by disruption in one or more elements of the learned fear process (Lissek and van Meurs 2014). Here, we will describe common laboratory-based measures of these processes, their relationship to symptom clusters and predictive validity for subsequent clinical trials if available, response to pharmacological treatment in both controls and PTSD patients, and considerations of their use in drug development studies.

2.1 Fear Conditioning and Cued Recall

Laboratory-based tasks to elicit Pavlovian fear conditioning in humans induce learned fear typically by presenting a visual conditioned stimuli (CS), such as simple shapes or images in combination with an aversive unconditioned stimulus (US) such as shock to the wrist or air puff to the throat. Operational measurement of fear responding to the CS+ (CS associated with US) is derived by comparing

behavior or physiological responses to the CS+ compared to CSs that are not presented with the US (i.e., safety signal, CS−) or when no cues are presented. Variations include examining responses to "contextual" versus discrete CS+ [to examine phasic versus sustained fear responses (Garfinkel et al. 2014; Glenn et al. 2014; Grillon et al. 2006)].

2.1.1 Do PTSD Patients Exhibit Increased Fear Learning/Expression? Is Fear Learning/Expression Related to Specific Symptom Clusters?

The short answer is it depends on the measure. PTSD patients exhibit increased potentiated startle responses to discrete fear cues (Briscione et al. 2014; Norrholm et al. 2011) and contextual fear cues (Grillon et al. 2009b); however, increased fear is not consistently detected using other behavioral or physiological measures such as self-report or skin conductance response (SCR) (Glover et al. 2011; Milad et al. 2008). This difference may be related to specific fear circuitry that is being probed by these behavioral measures, as startle reactivity is thought to reflect "automatic" fear conditioning processes that do not rely on contingency awareness, while SCR and self-report reflect fear processes that require contingency awareness (Jovanovic et al. 2006; Tabbert et al. 2006). Given that increased startle reactivity is commonly described by patients (DSM-IV, DSM-5), startle measures of fear may specifically probe abnormal circuits and mechanisms in PTSD that drive "automatic" fear responses (Grillon 2009). As might be expected, increased fear-potentiated startle is associated with high levels of re-experiencing symptoms in PTSD patients (Norrholm et al. 2011) and attentional bias to threat (Fani et al. 2012). However, in a study that directly compared fear acquisition across subjects with PTSD, general anxiety, or depression symptoms, increased fear expression was significantly higher in individuals with general cognitive and somatic anxiety symptoms rather than PTSD or depression symptoms (Acheson et al. 2015b). Greater conditioned fear expression has also been reported in other anxiety disorders, such as panic disorder (Grillon et al. 2008) and bipolar disorder (Acheson et al. 2015c). Thus, increased fear expression may reflect a biological abnormality in subpopulations of anxiety and mood disorder patients, crossing diagnostic classifications.

2.1.2 Is Conditioned Fear Responding Sensitive to Drugs that Are Effective for PTSD?

A reasonable question when considering a laboratory-based task for drug discovery is whether the task shows predictive validity for known therapeutic compounds. Unfortunately, there is disappointingly little work in this area. In healthy controls, fear-potentiated startle responses to cues with moderate contingency prediction which are thought to elicit sustained anxiety are attenuated by sub-chronic (2 week) SSRI treatment and acute benzodiazepine treatment, while cues with 100 %

contingency for the aversive US are not (Acheson et al. 2012b; Grillon et al. 2006, 2009a). Fear conditioning as assessed by skin conductance is unaffected by sub-chronic SSRI treatment (Bui et al. 2013). These data suggest that fear-potentiated startle has predictive validity as a laboratory-based measure of fear acquisition/expression for PTSD under certain conditions, particularly when cues elicit more prolonged anxiety-like responses which may be activating differential neural circuits [e.g., bed nucleus stria terminalis, for review see Avery et al. (2015) and Burghardt and Bauer (2013)]. Does this mean discrete fear conditioning tasks are not predictive for PTSD therapeutics? Perhaps, but an alternative explanation is that current treatments, which work in 50 % or less of the population (Berger et al. 2009), are unable to treat this particular facet of the disorder and thus are not useful positive controls. Further evidence for predictive validity for SSRI effects in patients is that acute SSRI treatment potentiates fear expression in conditioned fear models, similar to accounts of increased anxiety symptoms in patients in the initial phase of SSRI treatment (Garcia-Leal et al. 2010; Grillon et al. 2007; Silva et al. 2001). Effects of prazosin, used for treating nightmares in PTSD patients and which has some efficacy in animal models of conditioned fear responding, have not been studied yet in these human models (Do Monte et al. 2013; Raskind et al. 2013). This lack of data is partly due to the requirement for incremental dosing increases over weeks to reach therapeutic levels necessary for efficacy for treatment of nightmares in PTSD, reducing the feasibility of using this compound for validation studies. Effects of behavioral therapy on conditioned fear are also relatively untested. One small study found no significant reductions in potentiated startle to trauma-related cues after exposure therapy despite >50 % reduction in symptoms (Robison-Andrew et al. 2014); however, another larger study did find that exposure therapy reduced trauma-potentiated startle (Rothbaum et al. 2014). Overall, the evidence for predictive validity in terms of sensitivity to SSRI treatment is suggestive, but there are clear nuances to the parameters and dosing strategy that must be considered if these models are to be used.

2.1.3 Does Fear Conditioning Predict Treatment Response?

Again, there is very little work in this area. One small pilot study ($n = 9 - 10$/group) showed that only patients that show discrimination in SCRs between the CS+ and CS− respond to SSRI treatment (Aikins et al. 2011). These data support the speculation that cue discrimination may probe neural circuits that are responsive to SSRI treatment, but more research is needed to confirm this preliminary finding.

2.1.4 Is There Evidence for Fear Conditioning to Be an "Intermediate Phenotype" Associated with Genes that Confer Risk for PTSD?

There is some suggestion that genes that confer risk for PTSD are also associated either with heightened fear conditioning or with disruption in ability to inhibit

conditioned fear in humans [see next section below and see Skelton et al. (2012) for review of genetic approaches to fear learning phenotypes]. Examples are genes involved in noradrenergic (ADRA2B), serotonergic (SLC6A4), and catecholamine signaling (COMT), in cellular signaling pathways that support neural plasticity [PRKCA and WWC1; for review see Wilker et al. (2014)], and in genes involved in the neuroendocrine stress response [PACAP/PAC1, Ressler et al. (2011)] and opioid signaling (Andero et al. 2013). Thus far, however, only candidate gene studies have been conducted on fear acquisition and expression phenotypes, no genome-wide association studies have been published yet.

2.2 Fear Extinction, Reconsolidation, and Reinstatement

Fear conditioning is vital for survival, enabling threat prediction and consequent behavioral responses to avoid harm. As cues become less predictive of aversive stimuli, however, organisms adapt to this change with reduced conditioned responding termed extinction. The process of fear extinction is subserved by a hippocampal–amygdala–prefrontal cortex circuit, with the prefrontal cortex activation of inhibitory circuits in the amygdala resulting in reduced fear responses to previously learned fear cues (for review see Milad and Quirk 2012). Extinction does not modify or "erase" the original CS–US association, but instead represents new inhibitory learning that actively competes with the original excitatory CS–US associative memory (Bouton 1993). This hypothesis is supported by a number of return of fear phenomena including reinstatement of conditioned fear, in which following fear extinction a brief re-exposure to an unpaired US induces full recovery of the original conditioned fear response (Haaker et al. 2014; Myers and Davis 2002). Modification of the original fear memory can occur, however, via reconsolidation, a period in which a memory is activated and is thus transiently labile, thought to subserve an "updating" function [see following sections below for further details (Nader 2015)].

2.2.1 Do PTSD Patients Exhibit Changes in Fear Extinction Processes?

PTSD has been described as a disorder characterized by a failure in extinction. Most trauma survivors exhibit PTSD symptoms initially after the traumatic experience; however, over time most survivors (80–90 %) will return to normal functioning, while a small subset continues to exhibit robust, debilitating trauma memories that interfere with normal functioning (Rothbaum et al. 1992; Rothbaum and Davis 2003). Extinction is a critical component to the efficacy of exposure therapy for PTSD, which exposes the patient to trauma-related memories and/or cues both in the clinic and in vivo (Craske et al. 2014).

PTSD patients exhibit reduced fear extinction learning and retention in the laboratory, indicating that poor extinction of fear responses to trauma-related cues may be a mechanism underlying PTSD (Acheson et al. 2015b; Milad et al. 2008; Norrholm et al. 2011). In a recent comparative study across subjects reporting primarily PTSD, general anxiety, or depression symptoms, extinction deficits were only observed in subjects with PTSD (Acheson et al. 2015b), suggesting that poor extinction is specifically related to trauma-related symptoms as opposed to general symptoms of low mood or ruminative anxiety. PTSD patients also exhibit functional and structural abnormalities in the fear extinction network including the hippocampus, amygdala, and frontal cortex [for review see Acheson et al. (2012a), Shvil et al. (2013)]. During extinction learning, PTSD is associated with reduced activation of the ventral medial prefrontal cortex and increased activation of the amygdala and dorsal anterior cingulate, suggesting reduced inhibitory modulation by cortical inputs to fear circuits (Shvil et al. 2013). Twin studies suggest that poor extinction observed in PTSD is associated with symptom state, rather than a vulnerability trait for PTSD (but see Lommen et al. 2013; Milad et al. 2008), suggesting it could play a role in maintenance of PTSD symptoms once they emerge. Hence, pharmacological enhancement of the neuroplasticity of this circuit is of particular interest for novel therapeutic approaches to PTSD, particularly in conjunction with exposure therapy.

2.2.2 Pharmacological Approaches for Fear Extinction in PTSD

There has been an explosion of basic and clinical research on mechanisms of fear extinction, with a large literature on the cell signaling mechanisms that mediate and modulate fear extinction learning and recall. This literature has recently been comprehensively reviewed (Maren and Holmes 2015; Singewald et al. 2015); thus, here, we will focus on a brief synopsis of the use of d-cycloserine (DCS), as this treatment is the most advanced, providing a primer in the successes and difficulties of translating animal and preclinical findings in fear behavior to clinical treatment strategies.

The concept of developing adjunctive pharmacotherapies for cognitive or exposure-based therapies was largely driven by the work of Michael Davis and Kerry Ressler. They first showed that DCS, a partial NMDA receptor agonist, administered during extinction training resulted in enhanced fear extinction recall in animals. Subsequently, they showed that DCS administered during virtual reality-based exposure therapy for fear of heights significantly increased the therapy's efficacy in reducing phobia symptoms (Ressler et al. 2004; Walker et al. 2002). These seminal papers more than a decade ago led to a burst of activity across a number of disorders, showing initial increased efficacy of DCS treatment for exposure therapies for phobias, panic disorder, and obsessive compulsive disorder which has been confirmed by two meta-analyses (Bontempo et al. 2012; Norberg et al. 2008). "High-throughput" clinical trials have been developed to test efficacy of drugs for enhancement of exposure-based therapy (Rodebaugh and Lenze 2013; Rodebaugh et al. 2013). However, the translation to exposure therapy effects in

PTSD patients is less compelling. Four studies have examined DCS enhancement of exposure therapy, with either positive effects (Difede et al. 2014), equivocal, or marginal effects (de Kleine et al. 2012; Rothbaum et al. 2014), negative effects (Scheeringa and Weems 2014), or even deleterious effects (Litvin et al. 2007). These mixed results have suggested a number of potential issues that need consideration when designing treatment trials for DCS (and other putative extinction enhancing treatments): (1) are the effects of DCS more on *speed* of response rather than *magnitude* of response to exposure, two differing hypotheses that will require different experimental designs/analysis to probe efficacy; (2) what is the correct dosing/timing of treatment; (3) does DCS's cognitive enhancement promote inhibitory learning to the extinction context, which might subsequently contribute to contextual renewal of fear (Vervliet 2008); and (4) does DCS need to be targeted toward only the successful therapy sessions [for a detailed review, see Hofmann et al. (2015)]. This latter issue is because DCS is a broad cognitive enhancer, it can enhance both fear learning and extinction learning (Lee et al. 2006); thus, if the exposure session is unsuccessful in promoting extinction, it could instead promote reconsolidation (i.e., strengthening of conditioned fear to trauma memories and cues) that is then increased by DCS treatment. Thus far, however, predicting a "successful" session versus an unsuccessful one has been elusive. Alternatively, other groups are working to identify prescriptive variables that predict which subjects would most benefit from treatment, i.e., those with the most severe PTSD, specific symptom classes, or other traits (de Kleine et al. 2012, 2014).

It is worth noting that in humans, DCS has generally been found to be more efficacious in adjunct trials with exposure therapy in patient populations, compared to enhancing extinction of conditioned fear produced in the laboratory in healthy controls. One study (Kuriyama et al. 2011) out of 3 found DCS (and valproic acid) to enhance extinction. This study was the only one to utilize a reinstatement component, with DCS during extinction training affecting not within-session learning or recall, but instead suppressing reinstatement. DCS was ineffective in studies that limited their design to testing extinction acquisition and 24-h recall (Guastella et al. 2007; Klumpers et al. 2012). It has been suggested that this lack of translation of DCS effects on extinction in animals to extinction in healthy human subjects may be because extinction protocols in the laboratory are not probing "automatic" learned fear and extinction processes, but are instead governed by top-down executive functions (Grillon 2009). More recent studies, however, suggest that extinction in healthy controls is sensitive to putative extinction enhancing drugs such as cannabinoid receptor agonists and oxytocin (Acheson et al. 2013; Das et al. 2013; Eckstein et al. 2014; Rabinak et al. 2013), which suggests that these tests are "translational" in that they are sensitive to drugs that have shown efficacy in animal extinction studies (Singewald et al. 2015). Whether these drugs can then also make the leap to enhancement of exposure therapy or PTSD treatment is thus far mixed. Efficacy of cannabinoid receptor agonists for treating PTSD symptoms is promising (Cameron et al. 2014; Roitman et al. 2014), while oxytocin effects on exposure therapy are less clear (Acheson et al. 2013, 2015a; Guastella et al. 2009; Acheson and Risbrough 2015).

2.2.3 Is Fear Extinction Sensitive to Drugs that Are Effective for PTSD?

Although the bulk of pharmacology directed at extinction processes has been of drugs that are hypothesized to specifically act on this mechanism, it is fair to ask whether extinction is sensitive to current treatments. Chronic fluoxetine in rodents facilitates extinction learning and extinction memory recall, particularly in females (Deschaux et al. 2011; Fitzgerald et al. 2014; Lebron-Milad et al. 2013), and escitalopram enhances extinction in healthy humans (Bui et al. 2013), suggesting that examining effects of a drug on extinction may predict efficacy as an overall treatment beyond use as an adjunctive treatment with therapy. Paroxetine transiently enhanced effects of exposure therapy (Schneier et al. 2012); however, other studies show no efficacy of SSRIs to enhance exposure therapy in PTSD (Foa et al. 2005; Hetrick et al. 2010). It should be noted that when undergoing exposure therapy, many opportunities for exposure are outside of the therapist's office via "homework" developed to promote in vivo exposure in the patient's environment [in addition to imaginal exposure in prolonged exposure]; thus, a drug that can be given chronically may actually be more effective than a drug limited to exposure session treatments. Based on lessons learned from DCS in terms of potential unintentional enhancement of fear learning/reconsolidation, chronic treatment will depend on how selectively the drug acts on fear extinction mechanisms versus broader mechanisms of neural plasticity. (Besides its non-selective effects on extinction, DCS cannot be given chronically due to rapid tolerance.) An example of a potential target with more selective effects on extinction enhancement are agonists of the cannabinoid 1 receptor, in particular drugs that enhance endogenous ligand availability via inhibition of degradation (Steckler and Risbrough 2012).

2.2.4 Does fear extinction performance predict treatment response?

Currently, it is unknown whether extinction performance or other markers of extinction (e.g., ventral medial frontal cortex activation during recall) predict what type of treatment (e.g., pharmacology versus exposure therapy) or how much treatment (e.g., how many exposure sessions) might be most beneficial for patients. This question is of great interest in terms of supporting personalized medicine approaches and is actively being pursued by a number of research groups.

2.3 Reconsolidation and Reinstatement

Reconsolidation occurs when a memory is reactivated resulting in a period of transient lability of the underlying neuroplastic mechanisms supporting the

memory. During reconsolidation, old memories can be strengthened or disrupted by drugs that modulate consolidation mechanisms. The best characterized manipulation of reconsolidation of conditioned fear is via noradrenergic manipulations, with propranolol, a beta-adrenergic receptor antagonist, disrupting reconsolidation and subsequent conditioned fear responses in both animals and humans [for review see Otis et al. (2015)]. A recent meta-analysis indicates that propranolol is effective for blocking both consolidation and reconsolidation of fear memories in healthy humans (Lonergan et al. 2013). Recent studies however suggest that experimental design may be critical, with efficacy of propranolol given before memory reactivation having limited effect (Wood et al. 2015). Sevenster and colleagues showed that propranolol effects were only observable in conditions in which reconsolidation occurred under prediction uncertainty (i.e., the CS+ may or may not be followed by the US), suggesting that reconsolidation only occurs if the memory is actively being updated with new information (Sevenster et al. 2012). This group also cleverly showed that reconsolidation can be triggered not just by the specific CS+, but also by a semantically similar stimulus. Memory reactivation by semantically similar stimuli was sensitive to propranolol disruption (Soeter and Kindt 2015). This finding supports the feasibility of reconsolidation-based therapy, given the difficulty in accurately reconstructing trauma specific cues.

Reinstatement is when previously extinguished conditioned responding is "reinstated" after re-exposure to a US (Rescorla and Heth 1975). This phenomenon supports the now established view that extinction training does not "erase" the fear memory, but instead creates a competing CS–"No US" association with the original CS–US association. This CS–"No-US" association is further complicated by its dependence upon the extinction training context (Bouton 2014; Bouton and Todd 2014.) Studies of fear reinstatement in humans are relatively new and thus far primarily in healthy human controls (Dirikx et al. 2007; Hermans et al. 2005; Neumann 2008; Sokol and Lovibond 2012). Preliminary evidence suggests that cannabinoid receptor agonists given during or immediately after extinction training may suppress reinstatement (Das et al. 2013). There is an excellent review of current findings, methodology, and considerations for developing reinstatement protocols for drug development from the Lonsdorf laboratory (Haaker et al. 2014).

2.4   Contextual Modification and Generalization of Learned Fear and Extinction

Pavlovian fear conditioning occurs not only to discrete cues associated with a trauma, but also to the context in which a trauma occurs. The definition of what constitutes an associative context remains broad, but typically includes at least one of the following qualities: (1) unpredictable prediction of the US; (2) longer duration than a common discrete CS; and (3) complex, multimodal features. Contexts have been operationalized in numerous ways in laboratory tasks,

including the experimental setting itself, a virtual reality setting, pictures of rooms, and simple cues with an unpredictable US association (e.g., Alvarez et al. 2011; Armony and Dolan 2001; Bouton et al. 2006; Glenn et al. 2014; Grillon 2002; Effting and Kindt 2007; Neumann et al. 2007).

2.4.1 Do PTSD Patients Have Altered Contextual Fear Learning?

There is substantial research on contextual fear learning in animal models of PTSD (e.g., Daskalakis et al. 2013), though laboratory research on contextual learning in PTSD patients remains limited. Elevated startle response to unpredictable contextual threat has been found in PTSD patients (Grillon et al. 2009a, b). This finding suggests that PTSD patients may have elevated sensitivity to unpredictable threat, which contributes to sustained tonic "anxiety" responding, associated with activity in the bed nucleus of the stria terminalis (Walker et al. 2003).

Successful fear learning about multimodal contextual features depends upon configural processing in which a single configural representation binds together numerous co-occurring contextual elements (e.g., Rudy et al. 2004). Configural representation is a hippocampus-dependent learning process supporting identification of whether a context is similar ("pattern completion") or dissimilar ("pattern separation") to a previously encountered context. Impaired configural processing of a traumatic context has been theorized to contribute to contextual overgeneralization of fear experienced in PTSD (Acheson et al. 2012a, b; Glenn et al. 2014). Few, if any, studies have directly examined configural fear learning processes in PTSD patients. A fear conditioning study using two-dimensional images of similar-looking rooms as distinct contexts found that PTSD patients demonstrated poorer differentiation than healthy controls between threat versus safe contexts in contingency ratings (Steiger et al. 2015). The authors note that the contextual stimuli used in this study were relatively simple static photographs of rooms (hallway, library) so contextual differentiation in this task may not have required configural processing. For example, it would have been possible to distinguish between contexts by attending to a single contextual element (the presence or absence of books on the walls) without considering the overall configurations, meaning that this task did not necessarily evaluate hippocampus-dependent contextual fear learning deficits in PTSD. Configural learning deficits have been found in PTSD combat veterans, and their non-trauma exposed twins relative to non-PTSD combat veterans (Gilbertson et al. 2007), though this study utilized a "cube and paper test" which did not examine contextual learning in relation to fear conditioning.

PTSD patients have been shown to exhibit deficient extinction of contextual fear (Steiger et al. 2015). There is an extensive literature on contextual modulation of extinction and return of fear in patients with anxiety disorders (e.g., Vervliet et al. 2013) and some evidence of altered contextual modulation of extinction in PTSD patients (Rougemont-Bücking et al. 2011).

2.4.2 Do PTSD Patients Have Altered Generalization of Fear?

Generalization of fear is the process whereby conditioned fear responding occurs not only to stimuli directly associated with the US, but also to stimuli similar to the CS (e.g., Dunsmoor and Paz 2015; Dymond et al. 2014). Fear generalization is a particularly relevant process for PTSD as much of the fear experienced by PTSD patients is triggered by encountering generalization stimuli (GS) which act as reminders of the trauma due to similarity to the original conditional stimuli, rather than through encountering the actual stimuli directly involved in the trauma. Laboratory assessment of fear generalization typically includes two phases: (1) a standard differential fear conditioning phase involving both a CS+ repeatedly predictive of an aversive US and a CS− never paired with the US and (2) a generalization test measuring responding to GSs with varying levels of similarity or relatedness to the CS+. The CS+ and CS− in generalization tasks commonly differ along a particular observable gradient, such as size or color (e.g., small circle/large circle, black square/white square), but there has been extensive research on non-perceptual forms of generalization as well including category-based, semantic, and symbolic fear generalization [for reviews see Dunsmoor and Paz (2015), and Dymond et al. (2014)]. Through such methodology, a generalization gradient is generated, indicating the extent to which strong conditional responding occurs only to GSs very similar to the CS+ (steep gradient) versus responding to GSs with high and low CS+ similarity (shallow gradient).

Despite a robust literature on fear generalization and a sound theoretical basis for the relevance of generalization to PTSD, laboratory research on fear generalization in PTSD patients is extremely limited. Relative to healthy controls, PTSD patients as well as panic disorder and generalized anxiety disorder patients show shallow fear generalization gradients, indicating overgeneralization of conditioned fear (Lissek et al. 2010, 2014a; Lissek and van Meurs 2014). These data are in line with findings that subjects with PTSD do not show physiological discrimination between CS+ and CS− cues, even though they report contingency awareness perfectly accurately (Acheson et al. 2015b; Jovanovic et al. 2012). This deficit in "automatic" fear discrimination between safe and threat cues appears to be specific to PTSD symptoms compared to generalized anxiety or depression symptoms (Acheson et al. 2015b). Thus, pharmacological enhancement of cue discrimination may be an effective strategy for a number of anxiety disorders, not just PTSD.

Recent neural models of fear generalization identify hippocampal substrates involved in both pattern completion (CA3 region, involved in recognizing a GS as similar to previously encountered CS+) and pattern separation (i.e., dentate gyrus, involved in recognizing a GS as dissimilar from previously encountered CS+), while subregions of the central and lateral amygdala, the bed nucleus of the stria terminalis, and the ventromedial prefrontal cortex have been implicated in expression of generalized fear (Besnard and Sahay 2015; Dunsmoor and Paz 2015; Lissek et al. 2014b). It is noteworthy that models of pattern completion and separation in fear generalization are similar to hippocampus-centered models of contextual fear learning (Kheirbek et al. 2012; Rudy et al. 2004). Configural learning is

thought to encode complex, multimodal features of the trauma environment, however, while the term fear generalization is typically used in relation to discrimination across relatively simple stimulus gradients. Greater generalization of simple stimuli may be expected when configural learning of contextual information is impaired such that context learning must be learned through elemental representation, a learning process in which individual contextual elements are not bound together but independently associated with the negative outcome (Maren et al. 1997; Rudy et al. 2004).

2.4.3 Are Contextual Fear Learning and Fear Generalization Processes Sensitive to Drugs that Are Effective for PTSD?

No research to date has examined drug effects on contextual fear learning or fear generalization processes in PTSD patients, though preliminary experimental research suggests that acute glucose consumption may enhance retention of differential configural fear learning (Glenn et al. 2014). In healthy subjects, acute administration of 1 mg of the benzodiazepine alprazolam reduced sustained startle responding in both predictable and unpredictable "context" periods, but did not alter responding to discrete cues associated with predictable and unpredictable threat (Grillon et al. 2006). These findings tentatively suggest that acute benzodiazepine administration might reduce sustained contextual anxiety in PTSD patients, though they do not indicate treatment effects for sensitivity to unpredictable threat.

Findings from animal research are mixed regarding medication effects on contextual fear learning. One recent review concludes that both acute and chronic SSRI administration reduce plasticity in the hippocampus and decrease expression of contextual fear learning (Burghardt and Bauer 2013), while another review suggests that chronic antidepressant administration enhances configural learning processes through promotion of neurogenesis in the dentate gyrus (Castren and Hen 2013). Given the involvement of pattern separation and pattern completion in both fear generalization and contextual fear learning, there is reason to expect that drugs promoting neurogenesis in the dentate gyrus might be used to both improve configural learning of contextual information and decrease overgeneralization of feared stimuli in PTSD patients (Besnard and Sahay 2015; Castren and Hen 2013). No research has directly examined drug modulation of contextual fear extinction in PTSD, though it has been argued that DCS promotes contextual safety learning (Vervliet 2008; Woods and Bouton 2006). Theoretically, drugs that improve pattern completion and separation could be used prophylactically during or immediately following trauma to improve specificity of learning and prevent overgeneralization of contextual or discrete fear (Glenn et al. 2014). Conversely, such drugs may be contraindicated for use in conjunction with exposure therapy for PTSD and other anxiety disorders given concerns that greater contextual specificity of fear extinction learning increases the probability of contextually mediated renewal of fear (Bouton et al. 2006; Vervliet et al. 2013).

2.5 Practical Considerations When Using Learned Fear Processes as a Marker of Drug Efficacy

Because fear conditioning involves active learning, consolidation, and recall, treatment regimens will have critical consequences on how drug effects can be interpreted. Whether a treatment is hypothesized to block fear consolidation (i.e., potential utility as prophylactic) versus simply block fear expression (i.e., therapeutic utility) is a key component to appropriate study design. Sub-chronic or chronic dosing regimens are the norm for initial early phase studies. Animal studies of when the drug is most effective, either at blocking fear conditioning or at expression, are critical in planning interpretable fear conditioning studies across the dosing timeline (e.g., condition before or during dosing to test drug effects on expression versus conditioning, respectively). There is a similar issue for studies of extinction, with a note of caution from our own studies on oxytocin effects on extinction. To test the effects of oxytocin on extinction, we employed a common 2-day protocol; on the first day, fear conditioning was followed by drug treatment and subsequent extinction training trials, with the fear recall test 24 h later. We found a significant increase in extinction recall in the oxytocin group (i.e., less fear than placebo), suggesting a potential enhancement of extinction encoding/consolidation (Acheson et al. 2013). A recent study using fMRI with a very similar 1-day design of fear conditioning being followed by treatment and extinction training confirmed that within-session extinction could be enhanced by pretraining oxytocin (Eckstein et al. 2014). These findings supported subsequent examination of oxytocin to enhance extinction-based therapy. However, a preliminary study we conducted in spider phobia subjects indicated that oxytocin treatment has the opposite effect than expected, and it interfered with exposure therapy effects, with placebo treated subjects exhibiting better long-term reductions in phobia symptoms than the oxytocin-treated subjects (Acheson et al. 2015a). It is not clear whether this lack of translation is due to a potential design problem in the exposure therapy trial, including too short an exposure regimen (1 session), or whether our interpretation of oxytocin effects in laboratory-based tasks was erroneous. An alternate interpretation is that oxytocin treatment, administered soon after fear conditioning, could instead have disrupted consolidation of the fear memory (Acheson and Risbrough 2015). Thus, what was interpreted as effects on improving extinction training/recall may have actually been interfered with fear consolidation, and only a test design in which conditioning and extinction are separated more widely in time (i.e., 24 h) can be sure of the correct interpretation. A 3-day design, with conditioning, extinction, and recall on separate days, is of course more difficult in terms of retraining subjects; however, such a design will greatly enhance accurate interpretation.

An additional concern in terms of drugs effects on fear extinction is whether inhibitory learning processes are expedited (i.e., faster reduction in fear) or made more robust to relapse. It has recently been noted that in exposure therapy, the extent to which reductions in fear are long-lasting and resistant to relapse may be of greater clinical value than the sheer magnitude of decrease in fear (Vervliet et al. 2013).

This same consideration should be given to evaluating drugs targeting fear extinction, with designs that incorporate assessment of long-term recall and resistance to return of fear.

3 Summary

In conclusion, the use of laboratory-based measures of fear processes has offered the promise of exciting new targets for PTSD. Although the field continues to have gaps between findings in laboratory-based fear and effects in exposure-based therapy (e.g., DCS and oxytocin), parallel work in better defining DCS effects on fear processes and how these effects might both impede and facilitate exposure are currently underway. Using laboratory measures of fear learning processes to predict treatment response in patients is also potential evolution of the utility of fear-based tasks in informing treatment approaches. As discussed above, careful evaluation of study design and treatment approaches within the fear learning/extinction continuum will be critical in early-phase proof-of-concept studies. Designing studies with assessment of long-term recall/resistance to reinstatement will also be critical in evaluating drug effects either on fear consolidation (inhibitory) or on fear extinction (enhancement or improved generalization) for the chances of efficacy in the clinic.

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