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14. ABSTRACT
This study examined mechanisms of fear conditioning (FC), as an animal model of post-traumatic stress disorder, and also FC effects on drug reward. C57BL/6 mice were fear conditioned via repeated exposure to aversive stimuli (footshock co-terminating with a tone) or a sham procedure. Percentage of freezing is measured in a distinctive testing compartment with this auditory cue during trials. Conditioned fear was achieved within 3 shock exposures but could only be extinguished after 45 extinction trials (same context, no shock). To measure effects on drug reward in FC mice, subjects were exposed to a standard conditioned place preference (CPP) paradigm using morphine (10 mg/kg SC). After CPP training, both sham FC and FC mice showed an equal preference for the morphine associated side. In both fear and drug reward, classical conditioning links unconditioned drug or fear responses to associated contextual cues and result in enduring pathological responses to multiple stimuli but in this case did not interact with each other. A commercially available drug, sodium butyrate, did not alter the time course of fear extinction learning in mice. Extinction therapy countermeasures seek to reduce both fear and drug conditioned responses using a set of techniques in which patients are repeatedly, exposed to conditioned appetitive or aversive stimuli and future studies need to examine how different pharmacological treatment approaches in better extinction fear conditioned responses and drug reward preferences.

15. SUBJECT TERMS
post-traumatic stress disorder, fear conditioning, extinction, drug reward

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a. Pharmacology Biochemistry and Behavior: Volume 97, Issue 3, January 2011, Pages 619-625
b. Pharmacology Biochemistry and Behavior: 2011, in press

1. Introduction:

This novel translational proposal examined how repeated stressor exposure in an animal model produces learned fear responses which emulate post-traumatic stress disorder (PTSD) and how such fear responses enhance sensitivity to drug reward and addiction-related behaviors. In the Veteran population, PTSD and substance use disorders are frequently comorbid conditions. An estimated 20% of returning Operation Iraqi Freedom (OIF) Veterans have developed PTSD; substance abuse is also prevalent with 35% of OIF Veterans exhibiting alcohol abuse (Hoge et al. 2004). These comorbid disorders are often chronic and disabling, their etiologies and neural mechanisms are unknown and only partially effective treatments are available. This study will link PTSD-associated behaviors to subsequent addiction and will emulate aspects of the experience of traumatized Veterans.

Conditioned fear learning is one of the central features of PTSD as demonstrated by cue-induced re-experiencing responses (e.g. flashbacks) that are slow to extinguish in humans (Rauch et al., 2006). Fear conditioning develops when a cue or context is associated with an aversive stimulus such as a mild foot shock (an unconditioned stimulus or US). This sequence elicits a response (an unconditioned response or UCR) such as freezing behavior. Following repeated exposures, subjects learn that specific conditioned cues or contexts predict the aversive stimulus and presentation of those cues or conditioned stimuli (CS) elicit a conditioned fear response (CR). In this project, the development and expression of conditioned fear responses (vs. control conditions) were measured in a mouse model. Induction of fear conditioning is followed by measurement of sensitivity to drug reward using a conditioned place preference (CPP) model to morphine. Conditioned drug reward is a relevant model in addiction because environmental cues (e.g. a barroom) induce craving and persistent susceptibility to drug relapse. It was hypothesized that induction of learned fear responses would result in enhancement of drug reward as measured by CPP. Because of the convergence of brain fear and reward systems in the extended amygdala and its connections to the nucleus accumbens it was hypothesized that repeated activation of these neural systems during fear conditioning produces neuroadaptive changes that enhance sensitivity to drug reward. The implication is that changes in these neural systems underlie the high comorbidity of addiction in PTSD found in Veterans.

Previous studies suggest that several types of stress exposures enhance drug reward however the results are variable mostly because of the nature of these stressors (Lu et al., 2003). Extensive neuroscience research demonstrates a convergence of the neural pathways that underlie both stress- and addiction-related behaviors. However, this previous research does not address relationships between PTSD and addiction. PTSD results from specific conditioned cue and contextual fear responses that persist over time and not from generalized stress responses. Neurologically, PTSD is conceptualized as a fear-conditioning process that results from amygdalar hyperresponsivity (Rauch et al., 2006).

2. Body:

The overall hypothesis is that subjects undergoing repeated exposure to an aversive stimulus that is paired with neutral cues develop ongoing fear responses (freezing behavior) to these cues and enhanced approach responses to drug associated contexts. These fear responses are hypothesized to resist extinction. Fear conditioning is hypothesized to produce neuroadaptations in amydalar-accumbal pathways that result in enhanced sensitivity to drug reward as measured by increased place preference to morphine. Synaptic plasticity is found in these neural fear and reward circuits and thus drugs which enhance plasticity like histone deacetylase inhibitors (HDACi) might enhance plasticity induced extinction learning. This proposal examined whether HDACi sodium butyrate alters the extinction of fear conditioning. We examined whether fear conditioning alters sensitivity to opiate reward in fear conditioned mice (vs. controls) using a conditioned place preference model.

Objectives: The objective is to examine the effects of fear conditioning on subsequent opiate reward.
Aim 1- Utilize a mouse model of fear conditioning, compared to a control manipulation, to produce cue-induced fear responses (freezing behavior) resistant to extinction.
Aim 2- Examine sodium butyrate effects on conditioned fear responses and their extinction.
Aim 3- Examine alterations in sensitivity to opiate reward in fear conditioned mice (vs. controls) using a conditioned place preference model.

This PTSD Concept project provides evidence for more extensive testable hypotheses that relate fear conditioning to sensitivity to addiction. Future projects will examine a more extensive series of PTSD- and addiction-related behaviors and their time course for development and extinction. They will provide more detailed analyses of the pathways of neural activation and examine histone deacetylase inhibitors (sodium butyrate) more completely. This is a proof of concept proposal examining sodium butyrate's effect on the extinction of fear conditioning. Future studies cam examine sodium butyrate's effects after the expression of fear conditioning and its more direct relevance to treatment in the clinical setting. With such knowledge, dual use pharmacotherapy approaches can then be examined in humans.

Fear conditioning validation: C57BL/6 males mice (24-26g) will be fear conditioned in apparatus with sound attenuated boxes (Coulbourn Inc., Allentown, PA) equipped with infrared cameras for automated and observer scored quantification of freezing. ***Training sessions*** will be three minutes in length with a 10 kHz, 75 dB tone sounding continuously for the last 30 seconds of the session and co-terminating with a 2 second, 0.5 mA electric footshock. In order to insure robust fear conditioning, the sessions will be repeated three times over three separate days at 24 hour intervals. This training procedure results in cessation of all motor behaviors (freezing) in response to subsequent exposure to the tone cue in the absence of footshock (Radulovic et al., 1998). A ***conditioned freezing test*** will be performed in separate groups of mice 1, 8, and 16 days following training and consists of computer automated and blinded observer ratings of freezing over three minutes to assess the impact of context alone and context plus cue (10 kHz tone). ***Control mice*** will be exposed to the same procedures with the exception that no footshock will be administered on the training days. Sodium butyrate is hypothesized to enhance the extinction of fear conditioning *This proposal will test a safe and commercially available drug (sodium butyrate) with high translational utility for human studies.*

Morphine place conditioning: To measure effects of opiate reward in fear conditioned (vs. control) C57BL/6 mice, subjects from fear conditioning experiments (on the day after freezing test) will be trained in CPP procedures using morphine or saline. CPP boxes reside within sound-attenuated boxes equipped with photocell beams for automated quantification time of activity in each box. The flooring conditions in the CPP and fear conditioned environments all provide different tactile cues. Mice will be first allowed to explore all three CPP compartments for 15 minutes during a ***pre-conditioning test*** on day 1. Over the subsequent 4 days of CPP, mice will be administered saline alternating with morphine (10 mg/kg SC) prior to 50 minute conditioning sessions. To measure place preference on day 6 of CPP, mice will be allowed to explore all three compartments for 15 minutes during a ***post-conditioning test***. Mice will undergo 4 more days of saline and morphine (10 mg/kg SC) conditioning. Place preference will be measured again during a ***post-conditioning test*** on day 11. Control subjects will receive saline throughout. It is hypothesized that fear conditioned mice show more rapid development of opiate CPP (demonstrated on day 6) and/or exhibit an increase in the preference time for the morphine chamber (on days 6 and/or 11).

3. Key Research Accomplishments

- Mice acquired robust conditioned fear responses (60-80% freezing) after six pairings of cue/context with footshock relative to unshocked controls (10-20% freezing). Conditioned fear responses were gradually extinguished to produce 30-40% freezing. This extinction-induced decline in conditioned freezing was seen at the conclusion of 45 extinction trials.

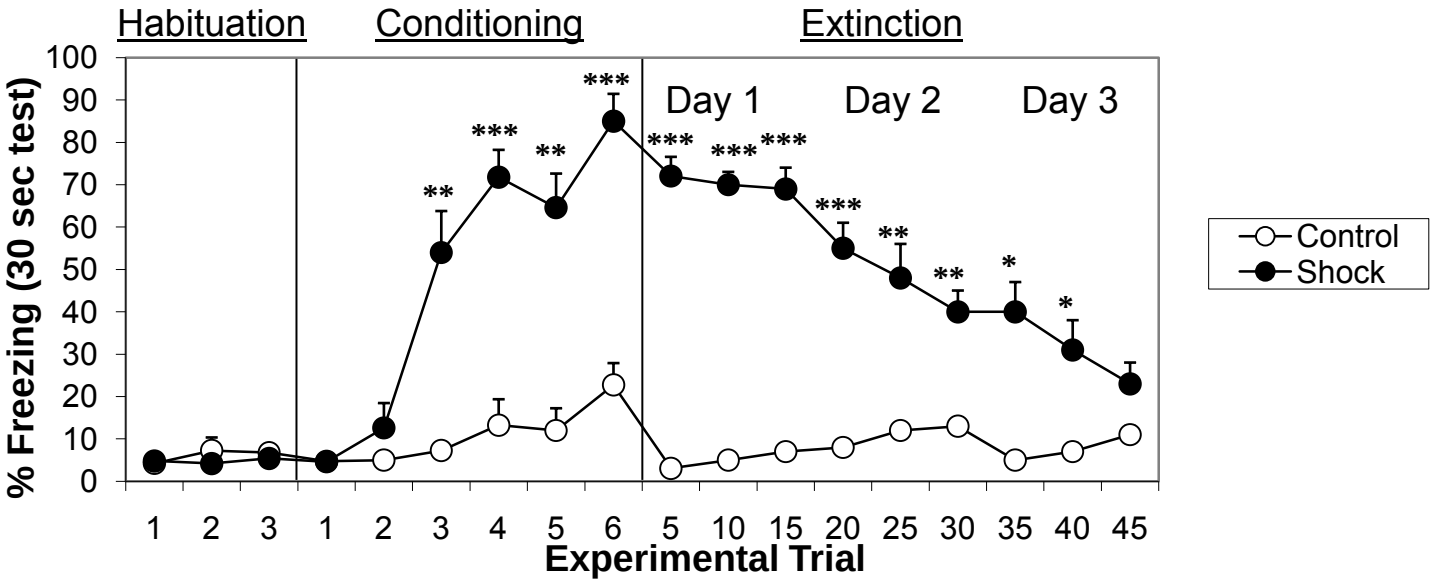
- Fear conditioning produced freezing responses to conditioned cues/contexts in rodents that were weakened most effectively by joint re-exposure to conditioned stimuli but were not enhanced by treatment with an HDAC inhibitor over a 12 day period. These studies suggest that extinction of an aversively conditioned response was not accelerated by enhancing chromatin remodeling during extinction training.

- To measure effects on drug reward in FC mice, subjects were exposed to a standard conditioned place preference (CPP) paradigm using morphine vs. saline conditioning. After CPP training, both sham FC and FC mice showed an equal preference for the morphine associated side.

4. Reportable Outcomes

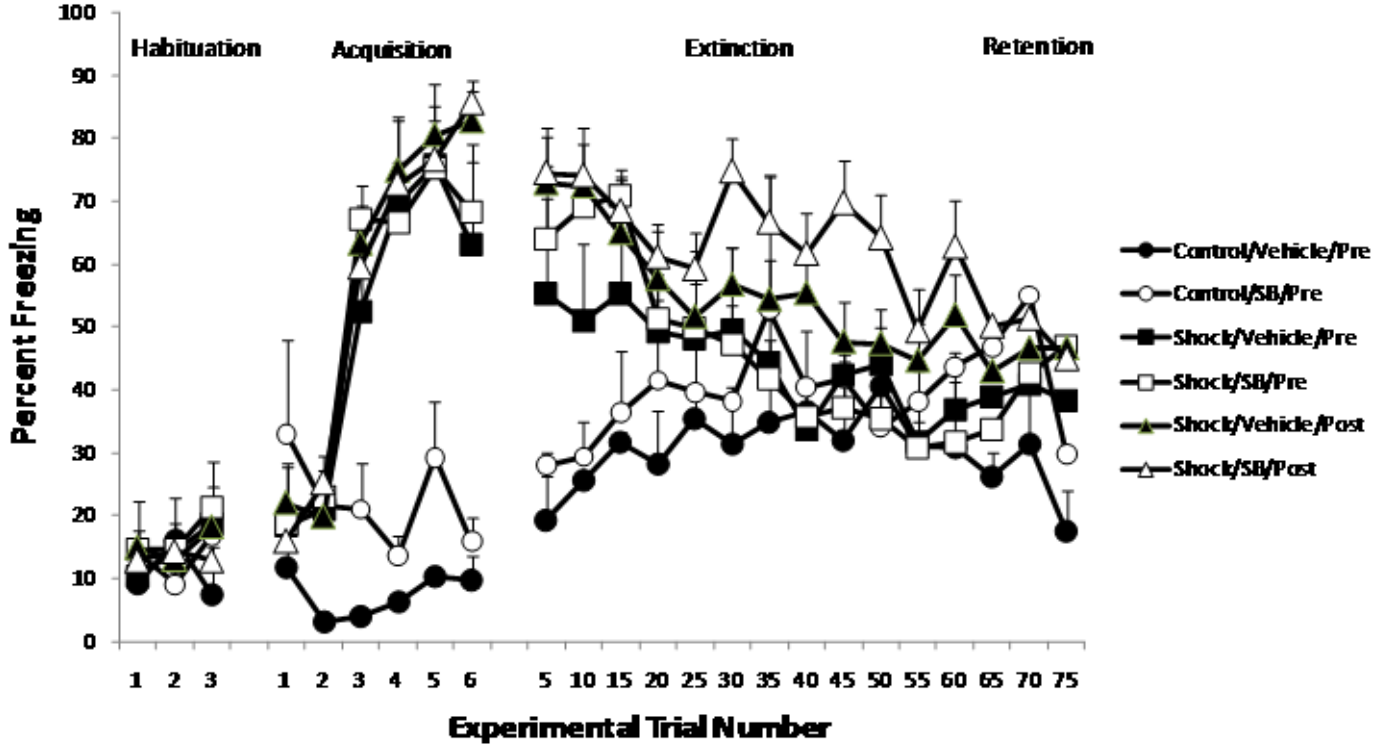
Fear conditioning in mice has been developed in Dr. Kaplan's lab using commercially available Video Fear Conditioning Chambers at the study site. These are computer interfaced enclosures equipped with shock generators and scramblers for administering electric current (US; 0.7 mA, 2 seconds) to the footpad. Chambers also contain tone generators for delivery of low-frequency tones (10 kHz, 75 dB) as an auditory conditioning stimulus. Footshock is initially paired with the tone cue, then the cue is delivered at later times in the absence of footshock in the same apparatus to measure cue and contextual conditioned freezing behavior. The dependent variable was automated conditioned freezing behavior after cue presentation. This measure was compared between mice receiving shock and control mice not exposed to shock which undergo the same procedures. **Figure 1** (next page) percentage of time spent freezing (mean + SEM) by mice in a distinctive testing compartment with auditory cues during habituation, conditioning, extinction, and spontaneous recovery trials. In Figure 1, mice acquired robust conditioned fear responses (60-80% freezing) after six pairings of cue/context with footshock relative to unshocked controls (10-20% freezing). Conditioned fear responses were gradually extinguished to produce 30-40% freezing. This extinction-induced decline in conditioned freezing was seen at the conclusion of 45 extinction trials.

Figure 1



In a second study (**Figure 2** below) Mice acquired robust conditioned fear responses (60-80% freezing) after six pairings of cue/context with footshock relative to unshocked controls (10-20% freezing). Conditioned fear responses were gradually extinguished to produce 30-40% freezing. This extinction-induced decline in conditioned freezing was augmented by sodium butyrate pretreatment over the final 7 days of training, with significant enhancement seen at the conclusion of 60 extinction trials. * $p<0.05$ relative to Shock/SB; + $p<0.05$ relative to Shock/Vehicle; SB – sodium butyrate.

Figure 2



Extinction of conditioned fear was hypothesized to depend upon chromatin remodeling which can be augmented pharmacologically using the histone deacetylase inhibitor, sodium butyrate. During extinction training, the conditioned freezing response was extinguished using 5 exposures/day to the tone cue/conditioning context in the absence of footshock following systemic pretreatment with vehicle or sodium butyrate (1 g/kg i.p.). Mice acquired robust conditioned fear responses (60-80% freezing) after six pairings of cue/context with footshock relative to unshocked controls (10-20% freezing). Conditioned fear responses were gradually extinguished to produce 30-40% freezing. This extinction-induced decline in conditioned freezing was not affected by sodium butyrate pretreatment over the final 7 days of training, with significant enhancement seen at the conclusion of 60 extinction trials.

This last study examined mechanisms of expression and extinction of fear conditioning (FC), as an animal model of post-traumatic stress disorder, and also FC effects on drug reward. C57BL/6 mice were fear conditioned via repeated exposure to aversive stimuli (footshock co-terminating with a tone) or a sham procedure. After training, mice demonstrated freezing behaviors over 80% of the time when exposed to the context and tone cues (vs. 10% freezing behavior in the control group). This was followed by fear extinction trials (20/day) via repeated exposure to context and cue without footshock; freezing behaviors returned to baseline levels. To measure effects on drug reward in FC mice, subjects were exposed to a standard conditioned place preference (CPP) paradigm using morphine (10 mg/kg SC) vs. saline conditioning. After CPP training, both sham FC and FC mice showed an equal preference for the morphine associated side.

5. Conclusions:

This study examined mechanisms of fear conditioning (FC), as an animal model of post-traumatic stress disorder, and also FC effects on drug reward. C57BL/6 mice were fear conditioned via repeated exposure to aversive stimuli (footshock co-terminating with a tone) or a sham procedure. Percentage of freezing is measured in a distinctive testing compartment with auditory cues during trials. Conditioned fear was achieved within 3 shock exposures but could only be extinguished after 45 extinction trials (same context, no shock). To measure effects on drug reward in FC mice, subjects were exposed to a standard conditioned place preference (CPP) paradigm using morphine (10 mg/kg SC). After CPP training, both sham FC and FC mice showed an equal preference for the morphine associated side. In both fear and drug reward, classical conditioning links unconditioned drug or fear responses to associated contextual cues and result in enduring pathological responses to multiple stimuli and in this case did not interact with each other. A commercially available HDACi drug, sodium butyrate, did not alter the time course of fear extinction learning in mice. Extinction therapy countermeasures seek to reduce both fear and drug conditioned responses using a set of techniques in which patients are repeatedly, exposed to conditioned appetitive or aversive stimuli and future studies need to examine how different pharmacological treatment approaches in better extinction fear conditioned responses and drug reward preferences.

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7. Appendices

Pharmacology Biochemistry and Behavior

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Treatment of Addiction and Anxiety Using Extinction Approaches:

Neural Mechanisms and their Treatment Implications

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25 pages (25 maximum), 4,600+ words (5,000 maximum), 2 figures (3 maximum), 63 references

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3. Integrated psychotherapy and pharmacotherapy to enhance extinction learning in addiction and anxiety disorders

4. Conclusions

Abstract (268 words, 300 maximum)

Clinical interventions which produce cue and contextual extinction learning can reduce craving and relapse in substance abuse and inhibit conditioned fear responses in anxiety disorders. In both types of disorders, classical conditioning links unconditioned drug or fear responses to associated contextual cues and result in enduring pathological responses to multiple stimuli. Extinction therapy countermeasures seek to reduce conditioned responses using a set of techniques in which patients are repeatedly, exposed to conditioned appetitive or aversive stimuli using imaginal imagery, *in vivo* exposure, or written scripts. Such interventions allow patients to rehearse more adaptive responses to conditioned stimuli. The ultimate goal of these interventions, extinction of the original conditioned response, is a new learning process that results in a decrease in frequency or intensity of conditioned responses to drug or fear cues. This review explores extinction approaches in conditioned drug reward and fear avoidance. The behavioral, neuroanatomical and neurochemical mechanisms of conditioned reward and fear responses and their extinction are derived from our understanding of the animal literature. Extensive neuroscience research shows that even though many mechanisms differ in conditioned fear and reward, converging prefrontal cortical glutamatergic pathways underlie extinction learning. Efficacy of pharmacological and behavioral treatment approaches in addiction and anxiety disorders may be optimized by enhancing extinction and weakening the bond between the original conditioned stimuli and conditioned responses. Adjunctive pharmacotherapy approaches using agents which alter glutamate or □-aminobutyric acid signaling or epigenetic mechanisms in prefrontal cortical pathways can enhance extinction learning. A comparative study of extinction processes and its neural mechanisms can be translated into more effective behavioral and pharmacological treatment approaches in substance abuse and anxiety.

Keywords (10 terms, 12 maximum): exposure therapy, extinction, substance use disorder, addiction, anxiety, posttraumatic stress disorder, classical conditioning, GABA, glutamate, dopamine

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Learning experiences involving drugs of abuse or fear responses are often associated with cue and contextual stimuli. Such stimuli in addiction include drug-related paraphernalia, money, and settings such as barrooms. Posttraumatic stress (or PTSD)-related stimuli include the people, places and things found in the accident area, war-zone, crime or abuse settings of the original trauma. Such experiences associated with these conditioned stimuli are stored as emotional memories. A period of transitory memory formation followed by a period of greater memory permanence suggests that memory traces become consolidated into the structure of the brain. Extinction learning is an active process that reduces the value or salience of these conditioned cues and contexts. Longer and repeated cue/contextual re-exposure without the associated fear or drug experience reduces conditioned responding. Extinction learning can be effective for reducing cue and context induced symptoms in addiction and anxiety and can improve outcomes in these disorders. The present review addresses the mechanisms of extinction learning and its translation into exposure treatment approaches. This comparative analysis may elucidate the neural mechanisms underlying extinction learning in these clinical disorders. Efficacy of pharmacological and behavioral treatments which enhance extinction learning can improve outcomes in substance abuse and anxiety disorders.

1. Conditioned drug reward and its extinction in addiction

1.1. Definitions of conditioned drug reward and its extinction

Drugs of abuse interact with stimuli in the drug-taking environment via classical and instrumental conditioning to alter motivational responses and self-administration behaviors (Robbins et al., 2008). Pavlovian conditioning mechanisms link unconditioned drug responses to associated contextual cues, allowing the drug responses to be elicited by these non-drug stimuli. For example, drug cue exposure in heroin and cocaine abusing individuals results in real-time drug craving

and consequent drug use (Epstein et al., 2009). The temporal and spatial relatedness of these contextual stimuli to motivational responses produce powerful conditioned effects (O'Brien et al., 1993). When these stimuli are encountered in an abstinent state, they can induce memories of prior drug experiences, or drug craving, which can result in drug taking and relapse (Weiss, 2005). Cue-induced craving is predictive of relapse in addiction. Any improvements in our understanding of mechanisms of craving or conditioned reward will lead to a better development of treatment approaches (Sinha and Li, 2007).

Animal models have been created to examine drug cue or contextual responses seen in human addiction. Several animal models demonstrate the powerful effects of drug cues or contexts on motivational responses. For example in the conditioned place preference model, an environmental context or cue is paired with experience of the drug (Sanchis-Segura and Spanagel, 2006). This conditioning results in the animal developing learning preferences for the drug associated contextual cue. Similarly, auditory or visual cues can be paired with drug self-administration and can be provided in relationship to operant responding (Sanchis-Segura and Spanagel, 2006; Nic Dhonnchadha et al., 2010). These cues act as conditioned reinforcers which increase behavioral responding for the drug.

Extinction is defined as a learning process that produces a reduction in the frequency or intensity of learned responses to conditioned drug cues. Extinction occurs after the removal of the unconditioned stimulus (UCS) that reinforced the learning in the conditioning environment (Franken et al., 1999). In place conditioning studies, extinction involves exposing rodents to the previously drug-paired context in a drug-free state (Epstein et al., 2006; Heinrichs et al., 2010). Extinction is an active process that results in the devaluation of conditioned stimuli. Repeated cue or contextual re-exposures without the UCS inhibits conditioned responding. However, following extinction of drug self-administration or place preferences, the presentation of drug associated stimuli can trigger renewed drug seeking behaviors and also serve as a measure of relapse and craving (Sanchez-Segura and Spanagel, 2006). Extinction is not simply a "forgetting" of conditioned behavior but instead is new learning process which acts to mask or inhibit original learning (Bouton, 2004).

Consolidation is a memory storage process occurring after a novel learning experience. Once a memory is stored, repeated presentation of the original conditioned stimulus (CS) initiates two processes, reconsolidation and extinction. The preservation of the original memory trace following initial retrieval is termed reconsolidation. Reconsolidation processes are most prominent when they are coincident with CS presentation and need to be masked in order for extinction learning to be efficacious. Thus, dual reconsolidation and extinction processes provide a framework for understanding and altering new memories. Defining the time course and duration of CS re-exposures in each of these

reconsolidation and extinction processes presents a challenge for the treatment of both addiction and conditioned fear (Taylor et al., 2009; Quirk et al., 2008; Tronson et al., 2008).

1.2 Neuroanatomical and neurochemical systems underlying conditioned reward and its extinction

The major components of the neural circuitry for cue and context induced reward and relapse include the prefrontal cortex, which includes prelimbic (PL) and infralimbic (IL) subregions, the basolateral amygdala (BLA), hippocampus, nucleus accumbens (NAc), ventral pallidum, and ventral tegmental area (VTA) as highlighted in Figure 1. Drugs of abuse produce direct motivational effects by activating dopaminergic neurons originating in the VTA which project to the amygdala, NAc, anterior cingulate cortex and PFC (Hammer Jr, 2002). Exposures to conditioned drug cue and context exposures also activate the mesolimbic dopaminergic system. In support of dopamine's role, cocaine dependent humans viewing drug cues demonstrate craving with correlated increases striatal dopamine levels (Volkow et al., 2006). In conditioned drug reward, classically conditioned cue and contextual responses are established via activation of neurons in the medial PFC (Taylor et al., 2009). Glutamatergic neurons from the prelimbic cortex (PL) of the PFC and from the basolateral amygdala (BLA) project to the NAc core region and to the VTA (Kauer et al., 2007) and are hypothesized to activate drug-seeking behavior (McFarland et al, 2003; Di Ciano and Everitt, 2004). Inactivation of these PL cortical neurons reduces relapse in rat models (LaLumiere and Kalivas, 2008). In contrast, glutamatergic projections from the infralimbic cortex (IL) to the NAc shell subregion are hypothesized to extinguish drug-seeking behavior (Peters et al., 2008; LaLumiere et al., 2010).

In addiction, the functional significance of each brain region is seen in Figure 1. The PFC is responsible for executive function, decision-making, and the implementation of goal-directed actions. Subregions of the PFC important in these functions the PL, IL and anterior cingulate cortex. The anterior cingulate cortex attaches motivational value to internal and external stimuli. The PL guides response initiation while the IL oversees response inhibition; both regions guide actions and outcomes. Several studies show that activation of the PL induces drug and fear responding while IL inhibits behavioral responding. These findings suggest that that PL-IL serve as on-off mechanisms in both conditioned drug reward and conditioned fear responding (LaLumiere and Kalivas, 2008; Peters et al, 2008; Quirk and Mueller, 2008; LaLumiere et al., 2010; (Weiss, 2005). The BLA processes appetitive and aversive stimuli and mediates approach or avoidance behavior. The hippocampus has a substantial role in cue and contextual associative learning and in memory consolidation and retrieval. The mesocorticolimbic dopamine projections that originate from the VTA are involved in the

initiation of motivational responses. The NAc integrates reward information from dopaminergic neurons and translates it to behavioral output regions through the ventral pallidum.

1.3. Translation of extinction learning into treatment for addiction

Drug dependent individuals demonstrate enduring cue and contextual reactivity even a year after intensive treatment, showing that cue reactivity is slow to extinguish. Increasing the rate and effectiveness of extinction learning is highly desirable in addictive disorders. One goal in the psychotherapy and rehabilitation of patients with substance use disorders is to impede reconsolidation of drug cues and/or facilitate extinction learning. Some support for the clinical efficacy of an extinction-related treatment approach has been demonstrated in abstinent cocaine addicts who were repeatedly exposed to drug-associated paraphernalia and outcomes were improved (O'Brien et al., 1993). Such an extinction based treatment is cue exposure treatment (CET). CET is a manualized presentation of drug-related cues and it has been shown to reduce cue activated emotional responses. This extinction treatment approach reduces craving and relapse but has been only partially effective in clinical trials. An early trial of CET in alcohol dependent patients utilized repeated exposure to the sight and smell of preferred drinks in a laboratory setting (Drummond et al., 1994). During a six- month follow-up, this CET treatment increased latency of relapse to heavy drinking and reduced alcohol consumption. In opiate addicted subjects, exposure of subjects to opiate related stimuli produced cue-induced negative emotions that also diminished after a cue exposure treatment protocol (Franken et al., 1999). This dampening of cue reactivity was maintained for at least 6 weeks after the last cue exposure therapy session.

However, subsequent trials of CET did not show similar efficacy. In a more recent trial, patients with alcohol dependence were assigned to either cue exposure or a standard cognitive behavioral treatment (Loeber et al., 2006). Both treatments reduced self-reported craving and increased self-reported measures of confidence to avoid relapse. However, relapse rates and other important drinking variables were not different at the 6-month follow-up in this last study. In a nicotine dependence treatment study (Niaura et al, 1999), cigarette smokers were assigned different conditions: (1) brief cognitive behavioral therapy (CBT); (2) CBT and nicotine gum; (3) CBT and cue exposure; and (4) CBT and cue exposure with nicotine gum. Abstinence rates were measured at 1, 3, 6 and 12-months post-treatment and the time to first slip. In this treatment study, there also were no differential treatment effects on abstinence rates or relapse times, suggesting no specific treatment efficacy. Other clinical trials (Marissen et al., 2007; Conklin and Tiffany, 2002) show that CET did not reduce relapse in substance use disorders and overall there is mixed efficacy of CET for relapse prevention. These studies suggest that additional psychosocial interventions such as cognitive reframing and motivational

enhancement might be necessary with extinction learning. Additionally, the specific elements of extinction such as timing and intensity of cue exposure, duration of treatment, and type of substance use disorder may affect outcomes.

A better understanding of the neural substrates underlying extinction will translate into treatment outcomes in addiction. In particular, pharmacotherapy can augment motivational and cognitive psychotherapeutic approaches in extinction learning. Future pharmacotherapy approaches may use glutamatergic, □-aminobutyric acid (GABA) receptor agents, cholinergic or other neurotransmitter receptor agents to enhance CET (Schroeder and Packard, 2004). For example, N-methyl-D-aspartate (NMDA) agonist D-cycloserine (DCS) has clear efficacy in enhancing extinction learning. In a meta-analysis of thirty animal and human DCS studies, Norberg et al. (2008) found that DCS significantly enhanced extinction in animals and exposure therapy in humans. In human addiction, DCS attenuated reactivity to smoking cues in nicotine dependent smokers (Santa Ana et al., 2009). In this double-blind, placebo-controlled pilot laboratory study, smokers were randomized to DCS or placebo, plus cue exposure therapy. DCS significantly reduced smoking cue reactivity in response to *in vivo* smoking cues as measured by physiological and urge cues. These preliminary findings provide support for DCS combined with cue exposure therapy in attenuating conditioned responses to drug cues.

Challenges exist in the use of pharmaceutical treatments as counter-conditioning treatments to block conditioned responses (CR) associated with drugs. One complication is that the pharmaceuticals introduce stimuli that can mask the CS so that it no longer evokes the CR. In treatment, the original CS may be present but the medication may substantially modify the interoceptive stimuli so that exteroceptive context no longer evokes the memory trace. Consequently, the blocking effects of a medication treatment are drug state dependent and as a result, the CS-CR bond remains unaffected. As a result, the conditioned drug reaction may be maintained once the pharmaceutical treatment is removed. When the CS is viewed as evoking the memory trace, then it is necessary to have the treatment implemented after the CS has activated the memory trace. At this time point, the treatment can potentially modify the memory trace directly and alter the CS-CR bond.

2. Conditioned fear response and its extinction in anxiety

2.1. Definitions of conditioned fear and its extinction

An extensive literature describes the phenomena of fear conditioning and its extinction in animal models and clinical anxiety disorders (Delgado et al., 2006; Quirk et al., 2008; Myers & Davis, 2007). Conditioned responding to aversive stimuli develops when a cue or context is associated with a threatening UCS. After repeated exposures, subjects learn that

a conditioned CS predicts aversive consequences and initiates a conditioned fear response (CR) such as freezing or avoidance behaviors (Radulovic et al., 1998). Pavlovian fear conditioning is an involuntary learning process that is long lasting and allows an organism to anticipate and prepare for potentially dangerous conditions (Maren, 2005). Fear conditioning is one of the central features of posttraumatic stress disorder (PTSD) as demonstrated by cue- and context-induced re-experiencing responses (e.g. flashbacks) or behavioral responses such as freezing (Quirk et al., 2008).

As in the reward, extinction of conditioned fear requires that the subject is repeatedly exposed to the CS after the removal of the UCS. Similarly, extinction learning results in a decrease in the frequency or intensity of conditioned fear. In fear conditioning, re-exposing the subject to the original CS produces one of two processes, reconsolidation or extinction of the CR (Peters et al., 2009; Quirk and Mueller, 2008). Through longer stimulus re-exposure or its repetition (without the UCS), extinction learning develops and conditioned fear responses decrease. The persistence of phobic and anxiety disorders may be caused by a failure to acquire or consolidate fear extinction (Rauch et al., 2006). There are also non-Pavlovian elements of extinction that modulate this learning including UCS devaluation and response fatigue (Myers and Davis, 2007).

2.2. Neural circuitry and mechanisms mediating fear conditioning and its extinction It is widely accepted that the amygdala, PFC, and hippocampus are key sites of synaptic plasticity and mediate the acquisition of fear conditioning (Corcoran and Quirk, 2007). Fear related sensory information is transmitted to the amygdala through its basal and lateral (BLA) nuclei. PTSD can be conceptualized as a cue- and context-associated fear conditioning process that results from amygdalar hyperresponsivity and an inability to extinguish these neural responses. The hippocampus is critical in associative learning, memory consolidation and in the retrieval of episodic memories. The sites of extinction learning may be distributed across several structures, especially the PFC and its corticolimbic projections to the amygdala (Figure 2). Glutamatergic excitatory projections from the PL extend to the sites of fear memory storage in the BLA and central nucleus of the amygdala (CNA) and activate downstream fear circuitry. Projections from the BLA to the CNA are thought to activate fear responses through outputs to the hypothalamus and brainstem. The IL cortex appears to be the primary candidate key pathway to suppress fear responses via extinction learning. Single unit recordings have shown that IL neurons respond to conditioned stimuli only after extinction learning has developed (Milad et al., 2006). Agents that activate IL pathways suppress conditioned fear (Vidal-Gonzalez et al, 2006). The IL projects to GABAergic neurons between the BLA and CNA called the intercalated cell masses (ITC) positioned between these two amygdalar subregions. Activation of the ITC inhibits output from the CNA and reduces fear responses. It has been hypothesized that fear

extinction entails an increase in excitatory drive to the ITC and produces reductions in output from the CNA (Peters et al., 2009).

As in addiction, consolidation of extinction learning involves NMDA receptor-mediated burst firing in the infralimbic (IL) portion of the PFC. Impairment of the ventromedial PFC by lesions or pharmacological inactivation reduces fear extinction and its retrieval (Burgos-Robles et al., 2007). Interference with PFC glutamatergic pathways via the administration of NMDA receptor antagonist drugs block the development or expression of fear extinction. The mechanisms underlying fear conditioning and its extinction have been studied most extensively in the thalamo- and cortico-amygdalar pathways where glutamate α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor subunits are involved. After extinction is consolidated and the fear CS is presented, cortical GABA is released and diminishes the firing of glutamatergic neurons that generate fear responses. Neuroimaging studies in humans also support involvement of both amygdala and PFC regions in fear extinction (Gottfried & Dolan, 2004).

2.3. Translation of extinction learning into treatment for anxiety

One effective psychotherapeutic approach for neutralizing fear conditioning in PTSD is prolonged exposure (PE) therapy, a form of extinction learning that has similarities to CET. Following the experience of a traumatic event many people can have persistent symptoms of arousal, avoidance and re-experiencing of the traumatic event. Such individuals experience distress and avoidance when confronted with thoughts, feelings, and situations related to the trauma. If trauma victims restrict their routines and avoid reminders of the traumatic event, then symptoms of PTSD are more likely to become chronic. PE produces extinction of trauma-related thoughts, feelings, and behaviors. The therapy utilizes a set of techniques in which patients use imaginal imagery, trauma-driven scripts, and *in vivo* exposure to reduce emotional reactions to feared objects and situations in a safe setting. Patients learn to understand that their fears and reactions to these stimuli are unrealistic (Foa, 2006). In a controlled study (Marks et al., 1998), patients with chronic PTSD were randomized to one of four treatments: PE (imaginal and live) alone; cognitive restructuring alone; combined PE and cognitive restructuring; or relaxation without PE or cognitive restructuring. Exposure therapy and cognitive restructuring therapy, alone or together, improved PTSD at the end of treatment and at follow-up. In a large randomized controlled trial of female veterans with PTSD, patients were randomly assigned to receive PE or control treatment (Schnurr et al., 2007). Women who received PE experienced a greater reduction of PTSD symptoms. The prolonged exposure group was more likely to no longer meet PTSD diagnostic criteria and to achieve remission. Thus, fear extinction therapies have been found to be efficacious in the treatment of PTSD (Foa, 2006). Similarly, treatment studies for specific phobias using

extinction training using *in vivo* exposure, desensitization, and virtual reality are acutely effective for different types of phobias (Choy et al., 2007).

3. Integrated mechanisms and treatment approaches to enhance extinction learning in conditioned fear and drug reward

Exposure therapies are effective in reducing PTSD symptoms (Foa, 2006) and partially effective in the extinction of craving and relapse in addiction (Conklin and Tiffany, 2002). Exposure therapies for both addiction and PTSD utilize imaginal approaches, written scripts, and sometimes live exposure techniques to reduce emotional reactions. Several factors constrain efficacy of extinction learning as presently implemented. The nature of the rewarding or adverse stimuli in addiction and PTSD, respectively, are different. In addiction, the sensory quality of the extinction contexts used in a treatment environment may only weakly approximate the reality of the drug-using environment. Of course, exposure to a naturalistic setting in addiction is potentially dangerous and cannot easily be employed in a treatment setting. Consequently, the efficacy of the extinction protocol is not sufficient to reverse the original conditioning so that extinction reminders are needed in treatment approaches. The relative permanence of the CS and CR bonds in addiction, and its resistance to extinction could account for the its partial efficacy in substance use disorders. Differences in reconsolidation and extinction learning may be operative in anxiety and addiction. Operant conditioning is likely to be relevant to addiction so that even if conditioned motivational responses are extinguished, the instrumental act of drug self-administration remains. Assisting addicted individuals with other interventions including cognitive, behavioral, motivational therapy and coping skills appear critical for maintaining abstinence (Rohsenow et al., 2001). The differing nature of rewarding and aversive stimuli and their conditioning mechanisms may produce the differential efficacy of exposure treatment in addiction and anxiety.

Extensive neuroscience research shows a convergence of evidence for the central role of PFC glutamatergic pathways in extinction of conditioned fear and conditioned drug reward behaviors (Peters et al., 2008; Quirk & Mueller, 2008; Taylor et al., 2009). In the medial PFC, the PL appears to serve as an on-switch for both conditioned reward and fear while the IL functions as an off-switch for both. This convergence is particularly striking when viewed in light of the fact that neural circuitry mediating reward (Figure 1) is different anatomically, neurochemically, and physiologically from substrates of fear conditioning (Figure 2). Thus, the use of pharmacological treatment approaches may optimize extinction learning. Adjunctive cognitive enhancement using pharmacotherapy approaches may improve the extinction of cue reactivity in addiction and PTSD (Cai et al., 2006). Agents that affect certain neurotransmitter mechanisms may be potentially useful in enhancing extinction. For instance, pharmacotherapy approaches with the N-methyl-D-aspartate

receptor partial agonist, D-cycloserine (DCS), and GABA receptor agents along with procedures derived from animal learning research can be more fully implemented in addiction and PTSD or phobia treatment (Berlau & McGaugh, 2006). DCS has been shown to enhance the extinction of fear conditioning and is hypothesized to hasten extinction of cocaine cue reactivity in animal models (Nic Dhonnchadha et al., 2010) and in humans (Price et al., 2009). Initial clinical trials demonstrate that DCS in conjunction with cue and contextual exposure therapies reduce anxiety for patients with social anxiety (Guastella et al., 2008; Hofmann et al., 2006) and phobias (Ressler et al., 2004). The $GABA_B$ receptor agonist, baclofen, improves hyperarousal and avoidance symptoms in chronic PTSD due to combat (Davis et al., 2003). $GABA_B$ agonists also have been shown to enhance the extinction of conditioned drug reward (Heinrichs et al., 2010). In a trial of baclofen for smoking reduction, treatment significantly reduced cigarettes smoked per day and drug craving (Franklin et al., 2009). The timing of drug administration is critically related to effects on memory has been shown to reverse the lowering of reward thresholds by drugs of abuse and reduce cue reactivity in addiction (Maccioni et al., 2008). DCS and GABA receptor agents show promise to enhance extinction learning in drug and fear conditioning and for increasing efficacy of exposure-based psychotherapy (Hofmann, 2007).

Finally, epigenetic mechanisms provide a novel avenue for altering gene expression underlying neural plasticity and behavior. Certain drugs modify transcriptional pathways through epigenetic mechanisms involving histones. Histones are highly basic proteins that organize DNA within the nucleus. Certain chromatin modifying enzymes, such as histone deacetylases, modify histone tails and in turn alter neuronal gene transcription (Roth and Sweatt, 2009). Treatment with histone deacetylase (HDAC) inhibitors induces dendritic sprouting, synaptic connections, and learning reinstatement (Fischer et al., 2007). Valproate modulates brain derived neurotrophic factor expression, increases long-term memory for extinction related to its HDAC inhibitor effects and enhances extinction training fear conditioned subjects (Bredy and Barad, 2008). HDAC inhibitor treatment facilitates extinction of cocaine-induced conditioned reward (Malvaez et al, 2010). In this last study, HDAC inhibitor treatment also increased histone acetylation in the nucleus accumbens following extinction. These results suggest a relationship between histone modification, epigenetic regulation of neurotrophic factors, enhancement of plasticity and and long-term memory for extinction of conditioned fear. These studies provide preliminary evidence that histone deacetylase inhibitors are agents for enhancing extinction learning in both addiction and conditioned fear.

4. Conclusions

In summary, learning experiences involving drugs of abuse or fear responses are associated with cue and contextual stimuli that produce conditioned responding to such stimuli. Extinction learning is an active process resulting after the devaluation of these conditioned cues and contexts. Extinction treatments have been preliminarily effective in reducing cue and context induced induced emotional and behaviors and improving outcomes anxiety and substance abuse disorders. Convergent neurobiological evidence documents the central role of PFC pathways in extinction of conditioned fear and drug reward behaviors. The essential goal of extinction therapies is to enhance long-term extinction responses by increasing resistance to spontaneous recovery, contextual renewal, and post-extinction reinstatement produced by re-exposure to conditioned cues or stress triggers. Future research and treatment needs to better incorporate all these elements into extinction learning. Additionally, comparative analysis of the neural substrates of original and extinction learning in reward and fear contexts provides significant potential for enhancement of therapeutic efficacy. Future pharmacotherapies may harness glutamatergic, GABAergic, or epigenetic mechanisms to facilitate cue and contextual exposure therapy efficacy. Such comparative studies of extinction processes and their neural mechanisms can guide the design of future clinical trials in substance use and anxiety disorders. Improvements in our knowledge of mechanisms relating to the expression and extinction of fear conditioning and drug cue reactivity will inform more comprehensive, standardized, evidence-based pharmacotherapy and behavioral therapies. Such knowledge can be translated into more effective treatment and will better allay the suffering of many afflicted individuals.

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The Use of Cognitive Enhancers in Animal Models of Fear Extinction

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Abstract

In anxiety disorders, such as posttraumatic stress disorders and phobias, classical conditioning links unconditioned fear responses to associated contextual cues resulting in enduring fear responses to multiple stimuli. Extinction is an active learning process that devalues these fear cues and contexts by weakening the bond between the original fear stimuli and conditioned stimuli. Extinction learning provides the basis of exposure-based treatments for human anxiety disorders. Extinction therapy is a set of procedures in which patients are repeatedly exposed to conditioned aversive stimuli in order to reduce negative conditioned responses. Since such psychotherapeutic approaches are not always effective, the animal research literature is reviewed to explore how cognitive enhancing pharmacological agents can improve behavioral approaches. Classical fear conditioning, inhibitory avoidance, and fear-potentiated startle paradigms provide animal models for the testing these cognitive enhancers in fear extinction. Extinction learning develops in these models after repeated conditioned cue or contextual re-exposure without the unconditioned fear stimulus. This review examines the effects on fear extinction of cognitive enhancers that act on gamma-aminobutyric acid (GABA), glutamatergic, cholinergic, adrenergic, dopaminergic, and cannabinoid signaling pathways along with agents that alter epigenetic and neurotrophic mechanisms. Of these cognitive enhancers, glutamatergic N-methyl D-aspartate (NMDA) receptor agonists, such as D-cycloserine, have reliably and dose-dependently enhanced fear extinction. Agents that function as glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor agonists, alpha2-adrenergic receptor agonists, neurotrophic factors and histone deacetylase inhibitors also improve fear extinction in animals but more confirmatory studies are needed. Such comparative studies of cognitive enhancers on fear extinction have important

implications for our understanding of extinction learning and can be translated into more effective pharmacological treatment approaches in human anxiety disorders.

Keywords (9 terms, 12 maximum): fear, anxiety, extinction, gamma-aminobutyric acid, glutamate, brain derived neurotrophic factor, amygdala, prefrontal cortex, plasticity

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1. Fear conditioning and its extinction in animal models

New pharmacological and psychological treatments can be targeted to the pathophysiological mechanisms underlying anxiety disorders such as posttraumatic stress disorders and various phobias. One important approach for novel pharmacotherapy for anxiety disorders is to improve learning that occurs in psychotherapy through cognitive enhancing agents. This review examines how such cognitive enhancers improve behavioral extinction approaches based on their effects in extinction learning in animal models of conditioned fear. The effects of cognitive enhancing GABAergic, glutamatergic, dopaminergic, noradrenergic, cholinergic, cannibinoid, and neurotrophic agents in animal models of fear extinction are reviewed. Such studies highlight the possibilities of combined cognitive enhancer treatment and psychotherapeutic approaches for humans with anxiety disorders.

1.1. Definitions of classical conditioning, memory consolidation and reconsolidation, and extinction

Classical conditioning develops when an organism is presented with an aversive or rewarding experience (unconditioned stimulus or UCS) that is paired with a cue (conditioned stimulus or CS) such as a light or tone or a specific learning context. After repeated pairings, a neutral cue or context (CS) starts to produce behavioral reactions (conditioned responses or CR) associated with the UCS. In fear conditioning, these responses include freezing behaviors and autonomic responses such as increases in respiration, heart rate, sweating, and pupillary dilation (Davis et al., 2006a). In the case of reward learning, conditioned cues or contexts associated with a drug of abuse elicits preference behaviors (Heinrichs et al., 2010). The temporal and spatial relatedness of the CS to the UCS produces powerful conditioned responses. As these learning experiences occur, new memories are stabilized by a process called consolidation. Memory traces are stored in neural structures as a result of the modification of synapses. However, retrieval of memory traces can induce an additional labile phase of learning. Thus, a period of transitory memories followed by a period of greater memory permanence and suggests that memory traces become consolidated into the structure of the brain (Tronson and Taylor, 2007; Taylor et al., 2009).

Once a conditioned response is consolidated, repeated presentation of the original CS initiates two processes, reconsolidation and extinction. Reconsolidation occurs when consolidated memories are stabilized after cued retrieval. Extinction is defined as a new learning process that results in decreased frequency or intensity of learned responses to conditioned cues. Extinction occurs after repeated CS exposure and the removal of the UCS that reinforced the learning in the environment (Tronson and Taylor, 2007; Taylor et al., 2009). In fear conditioning studies, extinction involves

exposing rodents to the previously fear-paired context without the UCS (Quirk et al., 2006; Peters et al., 2009). Extinction is an active process resulting from the devaluation of conditioned cues and contexts. Extinction is therefore not simply a "forgetting" of conditioned behavior but instead is new learning process which inhibits the original learning (Bouton, 2004). Reconsolidation processes need to be diminished in order for extinction learning to be effective. Defining the time course and duration of CS re-exposures in each of these reconsolidation and extinction processes presents a challenge for the treatment of conditioned fear (Taylor et al., 2009; Quirk et al., 2008).

1.2 . Animal models of fear conditioning and its extinction

The most popular animal models used to study the acquisition, expression, extinction, and reinstatement of fear include Pavlovian fear conditioning, inhibitory avoidance, and fear-potentiated startle paradigms (Shin and Liberzon, 2010). These models are presented so that effects of cognitive enhancers on fear extinction can be better understood. In Pavlovian fear conditioning, a neutral stimulus is contingently paired with an aversive UCS which activates innate fear behaviors such as freezing or autonomic responses (unconditioned responses or UCR). After conditioning, the CS comes to elicit various learned fear responses or CR's (Kim and Jung, 2006; Maren, 2008). Fear conditioning produces rapid, robust, and long-lasting learning. A single, intense footshock can produce conditioned fear learning in rodents that is retained for months (Maren, 2008). Pavlovian fear conditioning has primarily been employed using rodents (Kim and Jung, 2006; Maren, 2008) and in humans (Cheng et al., 2003; Alvarez, 2008). In rodents, behavioral responses involve behavioral suppression, motor freezing, analgesia, ultrasonic distress vocalizations and autonomic responses such as elevated heart rate, respiratory rate and blood pressure (Kim and Jung, 2006; Maren, 2008). In humans, changes in galvanic skin responding, heart rate, blood pressure, eyeblink conditioning and anxiety are often used as measures of conditioned fear (Shin and Liberzon, 2010; Cheng et al., 2008).

Extinction learning can be studied in fear conditioned animals after repeatedly exposing them to fear-eliciting CS in the absence of the aversive UCS. This extinction training results in a decrease in the amplitude and frequency of the fearful CR (Myers and Davis, 2002, 2007). This extinction procedure produces consistent results with both appetitive and aversive paradigms and across numerous species (Myers and Davis, 2002). The determining factor for the extinction of Pavlovian fear conditioning appears to be the violation of the expected contingency between the CS and the UCS (Myers and Davis, 2007). Pavlovian fear conditioning also allows the study of the reinstatement, renewal, and spontaneous recovery of learned fear. The reinstatement of fear responses refers to the reappearance of extinguished fear responses after UCS-only presentations. (Myers and Davis, 2002, 2007). Renewal of conditioned fear refers to the reappearance of

fear responses after extinction in a novel context and after a return to the original context (Myers and Davis, 2002, 2007). Spontaneous recovery refers to the reappearance of extinguished fear responses after the passage of time in the absence of any further explicit training (Myers and Davis, 2002, 2007).

Fear conditioning can also be acquired through operant conditioning paradigms in which the presentation of the aversive US is contingent upon the animal's behavior (Kim and Jung, 2006). Inhibitory avoidance occurs when an aversive stimulus such as a footshock follows an animal's behavior, such as moving to the dark compartment of a test chamber when placed in a lighted compartment or stepping down from a platform onto grid flooring. Following this conditioning, the animal learns to avoid performing the response that was followed by the aversive stimulus (Kim and Jung, 2006). Like Pavlovian fear conditioning, inhibitory avoidance procedures produce robust fear learning (Rossato et al., 2006). Typical extinction procedures involve repeated presentations of the CS in the absence of an aversive UCS which leads to the progressive extinction of the CR (Cammarota et al., 2003). Another model of anxiety, the fear-potentiated startle is a form of fear conditioning in which an aversive stimulus is associated with a neutral context or cue and results in an innate fear response, such as startle. After such conditioning, this CS alone can elicit the CR (e.g. startle response) that has previously linked to an aversive stimulus (for review see: Davis, 2006) and produces enduring effects (Campeau et al., 1990). The fear-potentiated startle model uses startle measurement as the dependent variable and this model has been well demonstrated in rodents (Chhatwal et al., 2005a; Walker et al., 2002) and humans (Norrholm et al., 2006). In fear-potentiated startle, the repeated presentation of the CS without aversive consequences results in a reduction in the frequency and amplitude of the CR (Walker and Davis, 2002).

Given these animal models of fear conditioning and their extinction, it is possible to examine pharmacological facilitation of extinction learning. The strategy is to examine neurotransmitters receptor and neurotrophic mechanisms that are involved in fear extinction and then test drugs that facilitate it. Using cognitive enhancing agents, multiple pharmacotherapies can be developed as adjuncts to exposure therapy (Davis et al., 2006a).

1.3. Neural mechanisms in fear conditioning and extinction

In order to effectively use cognitive enhancing drugs in fear extinction, it is critical to understand the key brain regions that are functionally involved. Animal studies across varied species and study paradigms have highlighted the importance of the amygdala in the acquisition and expression fear conditioning, the hippocampus in contextual fear learning, and the prefrontal cortex (PFC) in fear extinction (Shin and Liberzon, 2010; Maren, 2008; Pape and Pare, 2010). Using lesion-inactivation, stimulation, and pharmacological techniques, a large number of studies point to the amygdala

as one of the principal structures necessary for fear learning. This structure receives information about both unconditioned and conditioned stimuli and is responsible for activating an cascade of fear responses (Kim and Jung, 2006). Sensory information enters the basolateral nuclei of the amygdala (BLA) where new synaptic plasticity develops and produces CS-UCS associations. Inter-amgydaloid connections to the central nucleus (CNA), the primary fear output structure, allows the learned fear association to influence various autonomic and motor centers involved in fear responses (Kim and Jung, 2006; Davis, 2006a). Animal studies have also implicated the hippocampus as a vital influence in contextual fear conditioning. In rodents, hippocampal lesions impair conditioning to a contextual cue but not a discrete tone cue (Kim and Jung, 2006; Maren, 2008). Genetic studies have demonstrated the importance of the hippocampus in contextual fear learning using mutant mice with specific deficits in hippocampal long-term potentiation (LTP), a cellular model for plasticity and learning (Kim and Jung, 2006). Based on lesion studies, it appears that the hippocampus stores contextual fear memory using a transient temporal gradient (Kim and Jung, 2006; Maren, 2008).

The PFC is an essential brain region involved in the extinction of conditioned fear (Kim and Jung, 2006; Shin and Liberzon, 2010; Kaplan et al., 2010). The PFC is known to project to the amygdala, inhibit its neuronal firing and consequently reduce fear responding (Kim and Jung, 2006). The PFC inhibits the function of BLA by suppressing the conditioned fear responses after extinction training (Kim and Jung, 2006). The complexity of the connections between the medial PFC and BLA has lead to some controversy regarding the nature of PFC influences (excitatory vs. inhibitory) over the BLA (for review see Pape and Pare, 2010). The infralimbic cortex in the medial PFC is specifically involved in the consolidation of extinction learning and plasticity develops in this region for subsequent extinction retrieval (Mueller and Cahill, 2010). It has been recently been proposed that the neural circuitry underlying extinction of conditioned fear and drug seeking behaviors overlap in the PFC (Peters et al., 2009; Mueller and Cahill, 2010; Kaplan et al., 2010). For example, activation of the infralimbic PFC enhances extinction learning in both aversive (Mueller et al., 2008) and drug-seeking paradigms (Lalumiere et al., 2010).

2. Use of cognitive enhancers in fear extinction: pharmacological enhancement of fear extinction

This section examines the effects of GABAergic, glutamatergic, dopaminergic, noradrenergic, cholinergic, cannibinoid and neurotrophic agents as cognitive enhancers in animal models of fear extinction. The Table summarizes on the effects of these various cognitive enhancers on fear extinction from the animal research literature.

2.1 The role of gamma-aminobutyric acid (GABA) agents as cognitive enhancers in fear extinction

Many of the pharmacological interventions that facilitate conditioned fear and its extinction in animal models appear to do so by interacting with the major inhibitory neurotransmitter in the mammalian brain, GABA (Davis et al., 2006a). GABA agonists disrupt the acquisition of fear conditioning while GABA antagonists facilitate such acquisition (Davis et al., 2006a). Recent studies have examined the effects of GABAergic agents on fear extinction learning (for review see: Makkar et al., 2010). The evidence suggests that GABA agonists administered before or after extinction training inhibit consolidation of the extinction memory (Makkar et al., 2010). For example, benzodiazepines such diazepam or midazolam treatments, agents which enhance the effects of GABA binding at $GABA_A$ receptors, impaired extinction retention in a dose-dependent manner (Izquierdo and Pereira, 1989; Hart et al., 2009; Bouton et al., 1990). However, such effects on extinction were state dependent and did not alter the CS-CR bond (Izquierdo and Pereira, 1989; Harris and Westbrook, 1998; Davis et al., 2006a). Muscimol, another GABAergic agonist, infused directly into the dorsal or ventral hippocampus, before extinction training disrupted its retention (Corcoran et al., 2005; Corcoran and Maren, 2001; Hobin et al., 2006). In contrast, Akirav et al. (2006) found that infusions of muscimol into the BLA after extinction enhanced its retention. GABA antagonists have been shown to enhance cognition by blocking GABAergic transmission and are expected to facilitate extinction (Makkar et al., 2010). Post-extinction training administration of the GABA antagonists, picrotoxin or bicuculline, enhanced fear extinction (Berlau and McGaugh, 2006; McGaugh et al., 1990). However, in a series of experiments, Harris and Westbrook (1998) showed that $GABA_A$ receptor inverse agonist (an agent with opposite effects of the agonist), FG7142, slowed fear extinction and interfered with both the acquisition and expression of extinction learning. In summary, GABA agonists, antagonists, and inverse agonists have been shown to have complex effects on GABAergic transmission and extinction learning.

GABAergic neurons in the amygdala play a key role in both the expression and extinction of fear conditioning. PTSD can be conceptualized as a cue- and context-associated fear conditioning process that results from amygdalar hyperresponsivity. Fear related sensory information is transmitted through the BLA which connects to the central nucleus of the amygdala (CNA) and activates fear responses through outputs to the hypothalamus and brainstem. The infralimbic cortex appears to be the primary pathway to suppress fear responses via extinction learning (Quirk et al., 2008). The infralimbic cortex sends glutamatergic projections to GABAergic neurons between the BLA and CNA called the intercalated cell masses (ITC) (Likhtik et al., 2008). Activation of these ITC GABA neurons inhibits output from the CNA and reduces fear responses. Amano and coworkers (2010) showed that in fear extinction, increased GABA levels were found in CNA neurons along with enhancement of inputs to ITC cells during extinction training (Amano et al., 2010).

Extinction develops when pathways conveying sensory inputs about fear-eliciting cues in the amygdala develop experience-dependent forms synaptic plasticity (such as LTP) (Davis et al., 2006a). Expression of gephyrin, a $GABA_A$ receptor clustering protein, is downregulated in the amygdala after fear acquisition and upregulated after extinction training (Ressler et al., 2002; Chhatwal et al., 2005b). Fear conditioning produced decreases in amygdalar GABAergic plasticity which was measured by decreased mRNA expression of $GABA_A$ receptor $\alpha 1$ and $\alpha 5$ subunits and GABA synthesizing protein GAD67, as well as decreased benzodiazepine binding (Heldt and Ressler, 2007). In contrast, fear extinction produced increases in mRNA expression of $GABA_A$ receptor subunits $\alpha 2$ and $\beta 2$, and gephyrin and reduced GABA transporter-1 (Heldt and Ressler, 2007). Lin and coworkers (2009a) measured the effects of fear conditioning of miniature inhibitory postsynaptic currents (mIPSC) in the BLA and protein levels of $GABA_A$ receptor subunits. Fear conditioning decreased the frequency and amplitude of mIPSC and protein levels of gephyrin and $\beta 2$ while fear extinction reversed these effects. The effect of bilateral amygdalar infusions of an inhibitory peptide of $GABA_A$ receptors was measured in this last study. Blocking the insertion of $GABA_A$ receptors with this inhibitory peptide blocked fear extinction (Lin et al., 2009a). In summary, fear conditioning produces GABAergic synaptic plasticity in the amygdala as defined by downregulation of markers of GABAergic function while fear extinction produced an upregulation of these markers. Selective modulation of plasticity of GABAergic amygdalar neurons may ultimate prove useful in treatment of anxiety (Davis et al., 2006a).

2.2 The role of glutamatergic agents in fear extinction

The brain's major excitatory neurotransmitter, glutamate, has three major classes of receptors: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl D-aspartate (NMDA), and metabotropic glutamate receptors (Davis et al., 2006a). NMDA receptors are important in learning and memory and in experience-dependent forms of plasticity such as LTP. Administration of NMDA receptor antagonists before training blocks the extinction of the fear-potentiated startle response, contextual fear conditioning, inhibitory avoidance, and eye blink conditioning (Szapiro et al., 2003; Lee and Kim, 1998; Lee et al., 2006; Baker and Azorlosa, 1996; Falls et al., 1992). The use of NMDA antagonists immediately after extinction trials blocks extinction learning, suggesting that NMDA receptor activation is involved in consolidation of extinction (Santini et al., 2001). NMDA antagonists may block fear acquisition by disrupting glutamatergic transmission of sensory information to the amygdala (Davis et al., 2006a). Fear extinction may involve

experience-dependent plasticity between sensory pathways and GABAergic interneurons within the amygdala, suggesting a mechanism for NMDA receptor antagonist effects on fear extinction (Davis et al., 2006a).

Since NMDA receptor antagonists block fear extinction, many studies have examined the effects of cognitively enhancing NMDA receptor agonists, such as D-cycloserine (DCS), on fear extinction (Davis et al., 2006a). DCS binds to NMDA receptors as a partial agonist at the glycine site and enhances receptor efficacy by stimulating high-affinity glycine binding (Norberg et al., 2008). The promise of clinical translation using DCS is heighten by the fact that the drug has already been approved for human use by the FDA (Davis et al., 2006a). In a meta-analysis of animal and human studies using DCS, Norberg and coworkers (2008) summarized that DCS enhanced fear extinction in animals and also improved exposure therapy effects in humans with specific phobias, panic disorder, or obsessive compulsive disorder. At post-treatment, both animal and human studies were associated with moderate to large effect sizes (Norberg et al., 2008). Given the effects on fear extinction in both animal and human studies, DCS is a promising agent for improving exposure-based therapy outcomes in anxiety.

One study by Walker et al. (2002) showed that relatively high doses of DCS (15 and 30 mg/kg) given before extinction trainings enhanced the extinction of the fear-potentiated startle in rats compared to vehicle or low dose DCS (3.25mg/kg). Administration of a glycine site antagonist (HA-966) also blocked the DCS-induced enhancement of extinction. Extending these findings, Ledgerwood et al. (2005) found that DCS (2.5, 5, and 10mg/kg doses) given immediately after training dose-dependently enhanced extinction of conditioned freezing in rats. This finding suggests DCS mediates extinction by acting on memory consolidation after such training. Lee et al. (2006) compared the effects of systemic and intra-BLA administrations of NMDA agonist DCS and noncompetitive NMDA receptor antagonist (+)-5-methyl-10,11-dihydro-SH-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801) on fear extinction. When long extinction training sessions were used, MK-801 blocked extinction of conditioned freezing, and DCS potentiated extinction. However effects were reversed when shorter extinction training sessions were used. More research is needed to determine the long-term effects of DCS on fear extinction and to understand the optimal timing, number, and size of doses.

Evidence suggests that the effects of DCS decrease over repeated sessions (i.e. with chronic use) and that a single acute dose of DCS produces the greatest inhibitory effects (Norberg et al., 2008; Grillon 2009; Davis et al., 2006a and b). Since DCS plasma levels peak within hours after oral administration, maximal concentrations would develop during the period of post-session memory consolidation if given around a fear extinction or exposure therapy session (Davis et al., 2006a and b). Post-training administration of DCS might allow clinicians to give the drug after sessions in which with-in

session extinction occurred (Norberg et al., 2008). This would correspond to evidence in the animal literature which suggests that long-term DCS facilitation is only seen in animals showing within-session extinction (Norberg et al., 2008; Grillon, 2009). DCS was more effective when given a limited number of times and when administered close to the extinction training session. (Norberg et al., 2008; Walker et al., 2002; Ledgerwood et al. 2005). More research is needed to clarify whether DCS can be effective for individuals who did not respond previously to exposure therapy alone. Also it is unclear whether DCS is effective as a cognitive enhancer in a broader range of anxiety-related disorders (Norberg et al., 2008). DCS did improve patient outcomes for symptom severity, cognition, and functional impairment in social phobia when compared with placebo (Guastella et al., 2008).

Although DCS is the most studied cognitive enhancer its mechanisms of action are incompletely understood (Davis et al., 2006b). DCS appears to alter NMDA receptor mediated intracellular events such as calcium flux. Systemic DCS improved fear extinction when it was coadministered with intra-amygdalar injections of either protein synthesis inhibitor, mitogen-activated protein kinase inhibitor, a transcription inhibitor or a translation inhibitor, suggesting that these signaling pathways are critical to DCS as mechanisms (Yang and Lu, 2005). Fear conditioning is associated with AMPA receptor (GluR1) increases in the amygdala while DCS reversed fear conditioning and produced an internalization of GluR1 (Mao et al., 2008). These DCS effects were blocked by proteasome inhibitors. This suggests that DCS may induce the erasure of fear memory through GluR1 receptor internalization (Mao et al., 2008).

Modulation of glutamate AMPA receptors has been implicated as a mechanism in other fear extinction studies. Systemic and intra-medial PFC infusions of an AMPA receptor agonist, 4-[2-(phenylsulfonylamino)ethylthio]-2,6-difluorophenoxyacetamide (PEPA), facilitated fear extinction but had no effect on fear acquisition or consolidation (Zushida et al., 2007). The effects of PEPA on fear extinction were attenuated by an AMPA receptor antagonist drug pre-adminstration. Taken together, these results suggest that AMPA receptor agonist enhances fear extinction primarily through effects in the medial PFC (Zushida et al., 2007). Yamada et al. (2009) used different drug administration times and compared PEPA to DCS to clarify the role of NMDA receptors. They found that both agents enhanced the extinction of contextual fear learning but not its reconsolidation. Other AMPA receptor positive modulators have been shown to improve performance on several cognitive tasks (Woolley et al., 2009). AMPA receptor positive modulator, 5-(1-piperidinylcarbonyl)-2,1,3-benzoxadiazole (CX691) reduced conditioned fear suggesting that such compounds may be beneficial in the treatment of anxiety disorders (Woolley et al., 2009).

2.3 The role of cholinergeric agents in fear extinction

Nicotinic acetylcholine neurotransmission has been identified in enhancement of cognition and is a target for fear learning and fear extinction (for review see: Tinsley et al., 2004; Levin and Simon, 1998). Nicotine, the prototypical nicotinic acetylcholine agonist, has been shown to dose-dependently enhance cognition and contextual fear conditioning (Gould and Wehner, 1999; Gould and Higgins, 2003; Gould et al., 2004). Continuous nicotine treatment did not alter subsequent acquisition of fear conditioning or fear extinction, but enhanced the retention of fear conditioning (Tian et al. 2008). Nicotine has differential effects on fear extinction depending on when it is administered (Elias et al., 2010). Adolescent rats pretreated with low dose nicotine demonstrated enhanced fear conditioning compared controls and they failed to extinguish the fear responses (Smith et al., 2006). Nicotine administration given in proximity to fear conditioning may strengthen contextual fear memories and interfere with extinction.

Agonists at acetylcholine muscarinic receptors generally enhance memory and learning in animal models. Muscarinic acetylcholine neurotransmission has also been implicated in fear learning (for review see: Tinsley et al., 2004; Power et al., 2003). In general, muscarinic antagonists disrupt acquisition of new learning, while muscarinic agonists facilitate conditioning (Power et al., 2003; Tinsley et al., 2004; Rogers and Kesner, 2004; Gale et al., 2001; Soares et al., 2006). Cholinergic mechanisms within the BLA alter consolidation of extinction learning by modulating downstream GABAergic and noradrenergic signaling. Boccia et al. (2009) showed that intra-BLA infusions of muscarinic antagonist oxotremorine enhanced fear extinction when administered immediately after extinction training sessions. The effects of oxotremorine were not due to non-specific effects and the agent did not alter reinstatement of extinguished fear. Muscarinic antagonist agents have also been implicated in the recovery of conditioned fear after its extinction. Roldan et al. (2001) used a single-trial inhibitory avoidance protocol followed by extinction training. Muscarinic receptor antagonist scopolamine, given prior to retention testing, produced dose-dependent and time-dependent recovery of the previously extinguished avoidance response.

2.4 The role of adrenergic agents in fear extinction

Norepinephrine (NE) plays a critical role in attention, cognition and its extinction in PFC systems. Psychostimulants, such as adrenergic agent yohimbine, can enhance memory and learning. Although new memory consolidation is improved by noradrenergic signaling, some uncertainty remains as to whether NE enhances extinction memories (for review see: Mueller and Cahill, 2010; Davis et al., 2006a). Studies show that noradrenergic signaling modulates extinction in aversive, appetitive and drug-related learning paradigms (Mueller and Cahill, 2010). Systemic administration of noradrenergic drugs around fear extinction trainings yielded mixed results (Mueller and Cahill, 2010). Yohimbine, an

alpha2- receptor antagonist that promotes NE release, is one of the most well studied cognitive enhancers of fear extinction (Holmes and Quirk, 2010). Systemic administration of yohimbine facilitates fear extinction when training occurs in a context different from conditioning (Cain et al., 2004; Hefner et al., 2008; Morris and Bouton, 2007), but not when the context is the same (Mueller et al., 2009). Yohimbine-induced enhancement of extinction was dose-dependent and occured only with the administration of an optimal drug dose (Morris and Bouton, 2007). Yohimbine, when given in a neutral context, produces an extinction that was transferable to another setting (Morris and Bouton, 2007).

NE is critical to fear responses and administration of epinephrine resulted in the reinstatement of extinguished fear without reexposure to the dangerous context (Morris et al., 2005). Interestingly, post-extinction training using intra-amygdala infusions of NE facilitated the extinction of inhibitory avoidance (Berlau and McGaugh, 2006) showing the role of amygdalar NE in extinction learning. Fear-induced NE release in the amygdala may prepare this structure for subsequent consolidation of extinction. Administration of propranolol, a beta-receptor antagonist, has been shown to impair subsequent retrieval of extinction of contextual (Ouyang and Thomas, 2005), but not cued conditioned fear (Cain et al., 2004; Ouyang and Thomas, 2005; Rodriguez-Romaguera et al., 2009). In the cued fear paradigms, propranolol administration reduced fear expression during extinction training without affecting later extinction recall (Cain et al., 2004; Rodriguez-Romaguera et al., 2009). Pre-extinction session infusions of propanolol into the infralimbic PFC impaired the retrieval of extinction the next day (Mueller et al., 2008). Thus NE has both a critical role in the acquisition, expression, extinction and retrieval of conditioned fear.

Translational studies have examined the effects of adrenergic drugs for the treatment of clinical disorders in humans. Yohimbine has historically been used as a challenge procedure to induce anxiety among individuals with anxiety disorders (Mueller and Cahill, 2010). However, animal research has unexpectedly has found that yohimbine enhances extinction. Few clinical trials have examined yohimbine as a treatment in anxiety except for Powers and coworkers (2009) who used it in claustrophobic anxiety. Claustrophobia subjects took either yohimbine or placebo one hour prior to exposure to a small, dark, and enclosed space. Both treatment and control groups showed significant reductions in claustrophobic anxiety during the exposure. It should be noted that as a classic anxiogenic patients with PTSD, yohimbine induced flashbacks (Southwick et al., 1993) and panic attacks in those with panic disorder (Charney et al., 1987). Thus, yohimbine as a treatment for such patients would generally be contraindicated (Davis et al., 2006a).

2.4 The role of cannabinoid agents in fear extinction

The endogenous cannabinoid (CB) system has become a major focus in the search for pharmacological interventions

for fear extinction. (for review see: Davis et al. 2006a, Chhatwal and Ressler 2007; Varvel et al., 2009). CB1 receptor agonists and antagonists produce complex cognitive effects and alter extinction learning. CB1 receptors are involved in the processing of sensory information and in learning and are found at highest concentrations in the medial PFC, hippocampus, and BLA. Cannibinoid synaptic transmission is a source for plasticity in the form of long-term potentiation (LTP) in these regions. CB1 antagonist rimonabant (SR141716A) does not appear to affect the acquisition of cued fear conditioning (Mariscano et al., 2002) but does impair extinction learning in several protocols including fear-potentiated startle (Chhatwal et al., 2005a), auditory fear conditioning (Mariscano et al. 2002, Niyuhire et al. 2007), contextual fear conditioning (Suzuki et al., 2004), escape behavior in a water maze (Varvel et al., 2005), and passive avoidance of a foot shock (Niyuhire et al., 2007). Another CB1 receptor antagonist AM251 has been shown to have a mixture of effects on anxiety and conditioning. CB1 antagonists enhanced generalized fear (baseline freezing) and cued (CS) freezing. AM251 produced anxiogenic effects and increased baseline freezing while impairing extinction for baseline fear and cued fear conditioning (Reich et al., 2008). Bilateral infusions of AM251 into the CA1 region of the hippocampus after re-exposure to the conditioning context facilitated the reconsolidation of the fear memory, while the same local infusion blocked extinction learning (Alvares et al., 2008). Supporting these results were findings that extinction of fear conditioning was impaired in CB1 receptor knockout mice (Mariscano et al., 2002, Varvel et al., 2005, Kamprath et al., 2006). In contrast all of these findings, AM-251 reduced conditioned fear responding in a mouse model (Mikics et al., 2006).

If CB1 antagonism generally impairs fear extinction, it would be hypothesized that CB1 agonists should facilitate such extinction learning. There were mixed results with the administration of the CB1 agonist WIN 55,212-2. The CB1 agonist has been shown to increase the expression of conditioned fear (Mikics et al., 2006) and does not appear to affect fear extinction (Chhatwal et al., 2005a, Pamplona et al. 2006). However, systemic administration of a low dose of this CB1 agonist did facilitate extinction in other studies (Pamplona et al., 2006 and 2008). The timing and location of drug administration is critical as infralimbic infusions of WIN 55,212-22 prior to extinction training facilitated the extinction of fear-potentiated startle (Lin et al., 2008, 2009b). Chronic administration of this drug prior to fear conditioning attenuated the facilitating effect of pre-extinction training (Lin et al., 2009b). Improvements in fear extinction have been demonstrated with administration of AM404, an inhibitor of cannibinoid breakdown and reuptake. AM404 has been shown to have anxiolytic effects, decreasing anxiety in defensive startle, elevated plus-maze, and ultrasonic vocalization tests (Bortolato et al., 2006), without effecting baseline startle (Bortolato et al., 2006; Chhatwal et al., 2005a). Infralimbic infusions of AM404 after training reduced the fear-potentiated startle (Lin et al., 200b9). Using a fear-potentiated startle, AM404 produced dose-dependent enhancement of cued fear conditioning (Chhatwal et al., 2005a; Lin et al., 2009b) and

decreased shock-induced reinstatement (Chhatwal et al., 2005a). Systemic administration of AM404 or cannabidiol, a phytocannabinoid, given before extinction training extinguished fear responses (Bitencourt et al., 2008). AM404 and cannabidiol both have anxiolytic-like effects in naive and conditioned rats (Bitencourt et al., 2008). Chhatwal and Ressler (2007) showed that AM404 and similar compounds are anxiolytic and contrast with the majority of agents that enhance extinction and are often anxiogenic. Their anxiolytic effects make these agents especially attractive because they facilitate inhibition of fear through extinction-like processes and avoid the amnestic effects of many anxiolytics (Chhatwal and Ressler, 2007).

2.6 The role of dopaminergic agents in fear extinction

In general, activation of dopamine D1 and D2 dopamine receptors in the prefrontal cortex reverses cognitive deficits and enhances cognition in healthy subjects. However, these cognitive effects are task-dependent and as a result dopaminergic drugs produce complex and non-uniform effects. Both dopamine D1 receptor agonist SKF38393 and enhancers of dopaminergic efflux, amphetamine and cocaine, attenuated the extinction of a fear-potentiated startle response in rats (Willick and Kokkindis, 1995; Borowski and Kokkinidis, 1998). The injection of cocaine or SKF 38393 after extinction produced the renewal of fear-potentiated startle responses (Borowski and Kokkinidis, 1998). In a promising cognitive enhancement study, Dubrovina and Zinov'eva (2010) examined extinction of passive avoidance in intact mice and mice with depression-like "behavioral despair" presentation. In intact mice, activation and blockade of D1 receptors with SKF38393 and SCH23390, respectively, had no effect on extinction in the passive avoidance task. Activation of D2 receptors with quinpirole, but not blockade with sulpiride, led to a deficit in the extinction of the same task. In mice with the "behavioral despair" behaviors activation of D1 receptors with SKF38393 normalized extinction, while the D2 agonist quinpirole had no effect. The normalization of extinction was also produced with the blockade of both types of dopamine receptor by SCH23390 and sulpiride.

Modulation of dopaminergic systems in the medial PFC would be expected to modulate the rate of extinction (Quirk et al., 2006). In a review of extinction circuits involved in fear learning in the PFC, Peters et al. (2009) explained that the prelimbic and infralimbic cortices may provide a switch for expression of conditioned fear and its extinction, respectively (Peters et al., 2009). Microinfusions of the D1 receptor antagonist SCH23390 into the infralimbic PFC before extinction training impaired such learning while microinfusions of SCH23390 into the BLA caused impairments in fear acquisition (Hikind and Maroun, 2008). D1-receptor knockout mice also showed deficits in extinction (El-Ghundi et al., 2001). In contrast, systemic administration of D2 antagonist sulpiride to mice facilitated extinction of auditory conditioned fear

(Ponnusamy et al., 2005). Notably, sulpiride-treated animals demonstrated extinction to spaced presentations of the CS, a protocol that produced no extinction in vehicle-treated controls (Ponnusamy et al., 2005). In summary, cognitive enhancing dopamine D1 and D2 agonists appear to have mixed effects related to fear extinction.

2.7 Cognitive enhancers affecting epigenetic and neurotrophic mechanisms

Epigenetic mechanisms and neurotrophic factors represent novel targets for enhancing cognition and altering gene expression and associated plasticity. Certain drugs modify transcriptional pathways via histones which are highly basic proteins that organize DNA within the nucleus. Histone deacetylases are agents which modify histone tails and alter neuronal gene transcription. Histone deacetylase (HDAC) inhibitor treatment enhanced extinction training as it increased dendritic sprouting, synaptic connections, and neurotrophic factor expression (Bredy and Barad, 2008). Fear extinction was associated with histone acetylation around the BDNF gene promoter and also increased BDNF mRNA expression in PFC (Bredy et al., 2007). In this last study, HDAC inhibitor valproate enhanced fear extinction and synergized the behavioral effects of extinction training. Valproate enhanced the effects of extinction training on histone H4 acetylation around BDNF gene promoters and on BDNF mRNA expression. HDAC inhibitors sodium butyrate and trichostatin A produced greater effects in context-evoked fear extinction compared to vehicle control treatment (Lattal et al., 2007). There appears to be a relationship between histone modification, epigenetic regulation of neurotrophic factors, neural plasticity and fear extinction. HDAC inhibitors may be useful agents for enhancing fear extinction through specific plasticity mechanisms in the PFC.

Agents directly enhancing BDNF levels also produce increases in fear extinction via synaptic plasticity in the PFC. BDNF infused into the infralimbic PFC reduced conditioned fear in the absence of extinction training (Peters et al., 2010). These behavioral effects involved NMDA receptors and did not eliminate the original fear memory. Rats with impairments in fear extinction demonstrated reductions in BDNF levels in hippocampal inputs to the infralimbic PFC. By restoring BDNF levels in subjects with low hippocampal levels, extinction was improved. Mice expressing a human variant of BDNF demonstrated impaired fear extinction like the human phenotype (Soliman et al., 2010). This human phenotype showed impairments in frontal-amygdalar activity and this suggest the importance of BDNF targets in fear extinction. Mice with BDNF deletions also showed reduced fear extinction as demonstrated by fear-potentiated startle and freezing behaviors (Heldt et al., 2007). BDNF mediates its effects via tropomyosin receptor kinase B (TrkB) signaling. A lentivirus encoding a dominant-negative TrkB was used to antagonize BDNF signaling during fear extinction (Chhatwal et al., 2006). Lentivirus-infected rats showed impairments in their retention of extinction, suggesting that amygdala TrkB

signaling is necessary for the formation of stable extinction memories. In summary, these studies show that direct BDNF treatment or or its regulation by HDAC inhibitor treatment enhances infralimbic neural circuitry and increases fear extinction.

3. Conclusions

There is a wide array of cognitive enhancing agents which improve fear extinction. However, only a few of these agents produce reliable and dose-dependent effects in multiple models. Of all these cognitive enhancers, glutamatergic NMDA agonists appear to be most reliable for the enhancement of fear extinction across models. Other cognitive enhancers such HDAC inhibitors, BDNF/TrkB agents, glutamatergic AMPA agonists, and alpha-2 antagonist also enhance fear extinction, but more replications of these effects are necessary. A few other cognitive enhancers have produced mixed effects on fear extinction and more studies are needed to clarify the time- and localization-specific effects of administration of GABAergic antagonists, cholinergic nicotinic agonists, cannabinoid CB1 agonists, and dopamine D1 and D2 agonists. The adrenergic alpha-2 antagonist yohimbine has been shown to consistently facilitate fear extinction in animals (Cain et al., 2004, Hefner et al., 2008, Morris and Bouton, 2007; Mueller et al., 2009) and has been applied in translation research with humans (Powers et al., 2009). However, as an anxiogenic agent, the successful and safe use of yohimbine as a treatment for fear extinction is questionable (see for review: Davis et al., 2006a, Mueller and Cahill, 2010). The success of DCS as a fear extinction agent raises interesting questions about its neural mechanisms so that other similar agents can be pursued in testing.

NMDA agonist induced increases in synaptic efficiency in corticolimbic pathways via appears critical in mediating fear extinction. Intra-amygdala infusion of NMDA receptor antagonists blocks the development of conditioned fear and of LTP. This *in vivo* and *in vitro* research evidence provides a hypothesis that NMDA receptor-mediated LTP represents a cellular substrate for conditioned fear and extinction. Changes in plasticity in cortico-amygdalar pathways is mediated by AMPA receptor trafficking that appears critical to various aspects of conditioned fear. Enduring alterations in synaptic transmission depends on changes in the number and morphology of dendritic spines at cortical inputs to amygdalar neurons (Pape and Pare, 2010). The consolidation of fear extinction involves glutamatergic NMDA receptor-mediated burst firing in infralimbic portions of the PFC. Impairment of the medial PFC by lesions or by pharmacological inactivation reduces fear extinction and its retrieval (Burgos-Robles et al., 2007). Interference with infralimbic PFC glutamatergic pathways via the administration of NMDA receptor antagonist inhibits fear extinction and conversely

various NMDA agonists treatments (such as with DCS) enhance it. Mechanisms of fear conditioning and its extinction are most carefully studied at the plasticity in cortico-amygdalar pathways where glutamate AMPA receptor subunits are involved. Extinction training does not erase initial fear memories but rather leads to the formation of new inhibitory memories via changes in plasticity. Fear extinction appears to involve an inhibitory process competing with the original fear memories (Pape and Pare, 2010).

Some of the learning mechanisms which regulate extinction learning involve NMDA and GABA receptors, transcriptional regulation, and neurotrophic factor-induced increases in plasticity in cortico-amygdalar pathways. Extinction learning may reverse the synaptic changes induced by fear conditioning and result in physiological reversal of amygdalar LTP (Pape and Pare, 2010). DCS may trigger a signaling cascade resulting in AMPA receptor subunit internalization in the amygdala (Yang and Lu, 2005; Mao et al., 2008). The distinctive intra-cellular mechanisms of DCS offers one explanation for its unique success and points to new avenues of research in the search for cognitive enhancing agents. The synaptic mechanisms involved in the reversal of fear conditioning could be targeted with more specific pharmacological agents. A greater understanding of these neural mechanisms and pathways for fear extinction could aid in the search for new cognitive enhancers that facilitate this learning in anxiety disorders.

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Table- Effects of Cognitive Enhancers in Animal Models of Fear Extinction

Agent Class	**Drug**	**Fear Extinction Effect**	**Sources**
$GABA_A$ Receptor Antagonist and Inverse Agonist	Picrotoxin, Bicuculline; FG 7142	mixed results	McGaugh et al., 1990; Berlau and McGaugh, 2006; Harris and Westbrook, 1998
Glutamatergic NMDA Agonist	DCS	enhances	Ledgerwood et al., 2003, 2005; Woods and Bouton, 2006; Weber et al., 2007; Parnas et al., 2005; Walker et al., 2002; Lee et al., 2006
Glutamatergic NMDA Antagonist	AP5, MK-801, CPP	impairs	Falls et al., 1992; Szapiro et al., 2003; Lee and Kim, 1998; Lee et al., 2006; Baker and Azorlosa, 1996; Santini et al., 2001
Glutamatergic AMPA Agonist	PEPA	enhances	Zushida et al., 2007; Yamada et al., 2009
Cholinergic Nicotinic Agonist	Nicotine	mixed results	Smith et al.,2006; Tian et al., 2008; Elias et al., 2010
Cholinergic Muscarinic Agonist and Antagonist	Oxotremorine (agonist) Scopolamine (antagonist)	mixed results	Boccia et al., 2009; Roldan et al., 2001
Adrenoreceptor Alpha-2 Antagonist	Yohimbine	enhances	Cain et al., 2004, Hefner et al., 2008, Morris and Bouton, 2007; Mueller et al., 2009
Cannabinoid CB1 Agonist	WIN 55,212-2, AM404, Cannabidiol	mixed results	Chhatwal et al., 2005; Pamplona et al., 2006,. 2008; Lin et al., 2008, 2009; Bitencourt et al., 2008
Cannabinoid CB1 Antagonist	SR141716A, AM251	impairs	Chhatwal et al., 2005; Mariscano et al., 2002; Niyuhire et al., 2007; Varvel et al., 2005; Reich et al., 2008; Alvares et al., 2008
Dopaminergic D1 Agonist	SKF38393	mixed results	Willick and Kokkindis, 1995; Borowski and Kokkinidis, 1998; Dubrovina et al., 2010
Dopaminergic D2 Agonist	quinpirole	impairs	Nadar and LeDoux, 1999; Ponnusamy et al., 2005
Histone Deacetylase Inhibitors	Valproate, Sodium butyrate Trichostatin A	enhances	Bredy and Barad, 2008; Lattal et al., 2007; Bredy et al., 2007
BDNF/TrkB Agents	BDNF	enhances	Peters et al., 2010; Choi et al., 2010; Solimon et al., 2010; Heldt et al., 2007; Chhatwal et al., 2006