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REVIEW

Pharmacological enhancement of fear reduction: preclinical models

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Anxiety disorders have a high prevalence, and despite the substantial advances in the psychological treatment of anxiety, relapse is still a common problem. One approach to improving existing psychological treatments for anxiety has been to develop pharmacological agents that can be used to enhance the processes underlying exposure therapy, which is the most commonly used and empirically validated psychological treatment for anxiety during which individuals are taught to appropriately inhibit fear. Animal models of exposure therapy, particularly fear extinction, have proved to be a very useful way of examining the neural and molecular correlates of fear inhibition, which has in turn led to the identification of numerous drugs that enhance these processes in rats. Several of these drugs have subsequently been tested as novel pharmacological adjuncts to exposure therapy in humans with a range of anxiety disorders. The purpose of this review is to outline the key animal models of exposure therapy and to describe how these have been used to develop potential pharmacological adjuncts for anxiety disorders. Drugs that are currently in clinical use, as well as those currently in the preclinical stages of investigation, are described.

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Abbreviations

BDNF, brain-derived neurotrophic factor; CB1, cannabinoid receptor type 1; CS, conditioned stimulus; DCS, D-cycloserine; eCB, endocannabinoid; FGF2, fibroblast growth factor-2; HDAC, histone deacetylase; MB, methylene blue; mRNA, messenger RNA; NMDA, N-methyl-D-aspartic acid; PTSD, post-traumatic stress disorder; SSRI, selective serotonin reuptake inhibitor; US, unconditioned stimulus; WIN 55, 212-2, R-(+)-(2,3-dihydro-5-methyl-3-[(4-morpholinyl) methyl]1,2,3-de]-1,4-benzoxazin-6-yl)(1-naphthalenyl) methanone sulfonate

Fear is a natural, adaptive response to threat in the environment. However, when fear becomes persistent, even in the absence of danger, then this becomes maladaptive anxiety and impairs an individual's level of functioning. Anxiety disorders are the most commonly diagnosed psychiatric conditions in the western world with lifetime prevalence rates of approximately 30% in the USA and Australia (Kessler *et al.*, 2005; Slade *et al.*, 2009). Further, the World Health Organisation (2001) predicts that by 2020, mood and anxiety disorders combined will be the second leading cause of burden of all diseases (the most burdensome being heart disease). Clearly then, it is important to ensure that effective treatments are available to reduce the impact of anxiety disorders on society and the individual. One approach has been to examine the acquisition and reduction of fear responses in the laboratory using animal models with the aim of translating these findings to human populations with anxiety disorders. In recent years research has focussed on using animal models to develop pharmacological adjuncts that can enhance the processes underlying fear inhibition. The purpose of the current review is to describe how common animal models of fear inhibition have been used to develop pharmacological adjuncts for anxiety treatments, as well as to discuss the benefits and limitations of using these models to this end. Before reviewing current models of fear inhibition, we first briefly overview the commonly used rodent models of fear and anxiety (see Table 1 for a glossary of key terms used throughout this review).

Table 1
Glossary of key terms

Term	Definition
Cognitive-behavioural therapy (CBT)	A type of psychological therapy commonly used to treat mood and anxiety disorders in humans. CBT treatment of anxiety disorders involves a combination of exposure therapy and corrective learning. Individuals are exposed to anxiety-eliciting stimuli in the absence of aversive outcomes and erroneous beliefs regarding these stimuli are corrected.
Conditioned stimulus (CS)	A neutral stimulus (e.g., light or tone) that elicits fear responses only after it has been paired with an aversive stimulus (e.g. shock).
Elevated plus maze	An apparatus often used for assessing unlearned fear in rodents. The maze has four arms – two that are open and two that are closed. More time spent in the open arms indicates reduced anxiety.
Extinction	A procedure in which learned fear is reduced or inhibited through repeated presentations of the CS in the absence of the US.
Fear conditioning	An experimental procedure that involves pairing a neutral stimulus (CS; e.g. light) with an aversive stimulus (US; e.g. shock). Following such training, presentation of the CS elicits a variety of fear responses.
Fear-potentiated startle	A procedure used to measure fear whereby an animal or human exhibits a larger magnitude startle reflex in the presence of a feared cue (either learned or unlearned) in comparison with a neutral cue (or no cue).
Freezing	A defence response used to measure fear in rodents, and defined as the absence of all movement except that required for respiration.
Habituation	A decrement in responding to a stimulus as a result of repeated presentations of that stimulus.
Light/dark test	A procedure for measuring unlearned fear in rodents. One compartment of a chamber is illuminated while the other compartment is dark. More time spent in the light section is taken to indicate less fear.
Open field	A procedure that can be used to measure fear in rodents. The procedure involves placing rodents in a large, open apparatus and measuring exploration. More time spent exploring the centre of the apparatus indicates reduced fear.
Passive avoidance	A procedure for measuring learned fear in which an animal's latency to enter the area of a chamber in which it was previously shocked is recorded. Longer latencies indicate greater fear.
Reconsolidation	A procedure in which a reminder of an original experience is presented (e.g., a feared cue). Following this, the memory re-enters a labile state and must undergo a (re)consolidation process similar to that initiated when the memory was originally consolidated.
Renewal	The return of responding to a CS following extinction when testing occurs in a physical context different to where extinction training occurred.
Reinstatement	The return of fear after extinction produced by presentation of a stressor or unsignalled US before test.
Spontaneous recovery	The return of responding to a CS as the interval between extinction training and test increases.
Suppressed feeding	A response used to measure fear (learned or unlearned) whereby a rodent consumes less food in the presence of a feared cue in comparison with a neutral cue.
Unconditioned stimulus (US)	An innately aversive stimulus (e.g. foot shock, loud noise).

Models of fear and anxiety

Importantly, many of the fear responses seen in humans with anxiety disorders can be effectively modelled in rodents (see Cryan and Holmes, 2005 for review). The fact that there are a number of procedures that measure behaviours in the laboratory rodent that are akin to what is observed in anxious humans greatly enhances the translational potential of this work.

Learned fear

The most common technique for examining learned fear in the laboratory involves Pavlovian fear conditioning, which occurs when a neutral stimulus such as a light is paired with an aversive unconditioned stimulus (US) such as a footshock. Following this, presentation of the previously neutral stimulus (now the conditioned stimulus, CS) elicits a number of fear responses. In rodents, these fear responses include freezing, fear-potentiated startle, increased heart rate, increased blood pressure and the release of stress hormones (Davis, 1992; Carrive, 2000). Similar fear responses are also seen in humans in the presence of a CS that was previously paired with an aversive US (Milad *et al.*, 2006). Although Pavlovian fear conditioning can be seen as an ecologically valid model of some anxiety disorders, it is important to note that it can account for the aetiology of some anxiety disorders better than others. For example, while posttraumatic stress disorder

by definition develops following a significant traumatic event (American Psychiatric Association, 2000), other anxiety disorders such as specific phobias are not always linked to an explicit conditioning experience. Hence, the ecological validity of Pavlovian fear conditioning as a model of the aetiology of anxiety disorders across the board remains a controversy in the literature. Nevertheless, fear conditioning provides a useful model of the behavioural characteristics of anxiety disorders (e.g. Mineka and Zinbarg, 2006). Another commonly used model of learned fear is passive avoidance, in which an animal's latency to enter the area of a chamber in which it was previously shocked is taken as a measure of fear (see McGaugh, 2004, for a review).

Unlearned fear

Fear conditioning procedures require that an animal be trained to fear an initially neutral stimulus; the animal will not express fear responses to the stimulus in the absence of such training. Other procedures take advantage of animals' innate fear of ecologically relevant stimuli; these procedures do not require any training in order for the animal to express fear responses and so are termed measures of 'unlearned' fear. The open field is one such measure of unlearned fear, and involves placing animals in a large, open apparatus and measuring the amount of defecation/urination (Hall, 1934) or movement/exploration (Treit and Fundytus, 1988). Rodents naturally avoid bright open spaces and when placed in an open field typically spend more time in the periphery rather than the centre of the apparatus. The elevated plus maze and the light/dark test are additional measures of unlearned fear based on the premise that rodents tend to avoid bright and exposed areas. The elevated plus maze has four arms (two open and two closed) and rodents usually avoid the open arms (Hogg, 1996). A greater proportion of time spent in the centre of the open field or in the open arms of the elevated plus maze is taken to indicate reduced anxiety (Cruz *et al.*, 1994; Prut and Belzung, 2003). In the light/dark test rodents are placed in chamber that has a well-illuminated section and a darkened section. Increased time spent in the illuminated section, and increased crossings between the sections, are taken to indicate reduced anxiety (Crawley and Goodwin, 1980). Other means of measuring unlearned fear include responses that are routinely used to measure learned fear. Importantly, it is the stimulus presented that distinguishes between learned and unlearned fear paradigms. For instance, a rat will exhibit high levels of freezing in response to a tone previously paired with shock (i.e. learned fear), but will also freeze in the presence of a predator (i.e. unlearned fear) (Corcoran and Quirk, 2007). Similarly, fear-potentiated startle can be used to measure unlearned fear whereby the acoustic startle response increases in the presence of an innately feared stimulus. In rats, startle is potentiated in a bright room (due to rats' innate fear of the light; Walker and Davis, 1997) and in humans startle is potentiated in a dark room, an effect that is correlated with the intensity of participants' fear of the dark during childhood (Grillon *et al.*, 1997). For a review of how the potentiated startle response can be used to measure both learned and unlearned fear see Davis 2006. Finally, another means of examining unlearned fear is the novelty suppressed feeding test, in which rats are deprived of food and then placed in a novel environment containing food. Increased feeding latency is taken as an index of increased anxiety (Bodnoff *et al.*, 1988).

Collectively, these procedures have been used extensively to examine the effect of pharmacological agents on state/trait anxiety. However, as pointed out by Cryan and Holmes (2005), models of anxiety based on approach/avoidance conflict do not differentiate between increased anxiety versus increased novelty-seeking/impulsivity, or changes in motor activity. Interpretations from such tests must be made with caution, and preferably in the context of a wider battery of tests.

Models of anxiety disorder treatment

Individuals with anxiety disorders are often unable to extinguish or inhibit fear responses (e.g. Guthrie and Bryant, 2006; Blechert *et al.*, 2007; Michael *et al.*, 2007; see Lissek *et al.*, 2005, for a meta-analysis). Therefore, behavioural treatments for anxiety disorders attempt to enhance the inhibition of fear in these individuals. The most empirically supported treatment for anxiety disorders is exposure therapy, in which individuals are gradually exposed to anxiety-eliciting stimuli in the absence of any danger or negative consequences. Commonly, individuals with anxiety disorders overestimate the probability and the cost of negative outcomes occurring. As such, exposure therapy involves two components, one that targets irrational beliefs about the probability of a feared outcome and a component that targets the cost of the feared outcome. Through this process, individuals learn that the chance of the feared outcome occurring is unlikely, and if it does occur, that this will not be as catastrophic as initially thought.

Although exposure therapy for anxiety disorders is a very effective treatment, not all individuals respond and relapse is a common problem, which means there is considerable room for improvement (e.g. Brown and Barlow, 1995; Hofmann and Smits, 2008). One approach to developing new ways to enhance the effectiveness of exposure therapy involves studying ways to facilitate fear inhibition in the laboratory using rodent models. To this end, any procedure focusing on the reduction or inhibition of fear may provide useful information that can be translated to humans with an anxiety disorder. There are a number of laboratory procedures that result in a reduction in learned fear responses as outlined below.

Extinction

Once an animal has been trained to fear a CS, its fear can be inhibited by a process called extinction. During a typical extinction procedure, the animal is repeatedly presented with the feared CS but in the absence of any aversive outcome. During extinction training, the animal gradually learns that the CS no longer predicts the US, and as such, their fear responses decrease over repeated trials. When tested with the CS the following day, the animal typically exhibits long-term memory for extinction, indexed by reduced levels of conditioned fear responding. However, there are a number of situations that result in recovery of conditioned responding to the CS following extinction training. For instance, presentation of an extinguished CS in a different physical

context to where extinction training occurred can result in a recovery of conditioned fear responses, a phenomenon termed renewal (Bouton and Bolles, 1979a). Conditioned fear responses can also recover when an unexpected aversive event (e.g. a mild footshock) occurs following fear extinction, a phenomenon termed reinstatement (Bouton and Bolles, 1979b). Finally, responding to the CS can be restored when extinction training and test are separated by a considerable passage of time, a phenomenon referred to as spontaneous recovery (Quirk, 2002). Given that exposure therapy was based on the extinction procedure (Wolpe, 1954; Meyer, 1957), it provides a useful preparation to test potential pharmacological adjuncts for use in treating anxiety disorders. Moreover, procedures such as renewal, reinstatement and spontaneous recovery can be used to model the return or relapse of fear following successful exposure therapy. Indeed, renewal (e.g. Milad *et al.*, 2005; Effting and Kindt, 2007), reinstatement (e.g. Hermans *et al.*, 2005; Norrholm *et al.*, 2006; Schiller *et al.*, 2008), and spontaneous recovery (e.g. Schiller *et al.*, 2008) have been demonstrated using fear conditioning and extinction procedures in non-clinical human participants. Additionally, the return of fear following exposure therapy for anxiety disorders fits into the same conceptual framework as the return of fear in rodent laboratory experiments (Rachman, 1989). For example, results showing relapse when patients are tested at follow-up periods can be classified as an example of spontaneous recovery (e.g. Philips, 1985; Brown and Barlow, 1995). Similarly, studies showing that negative life stressors are associated with poorer treatment outcome and attenuate treatment gains may be clinical examples of reinstatement (e.g. Steketee, 1993; Wade *et al.*, 1993). Finally, renewal effects might also be clinically relevant based on studies showing that testing phobic participants in a non-treatment context results in higher levels of fear compared with groups that were tested in the treatment context (e.g. Rodriguez *et al.*, 1999).

One of the limitations of drawing conclusions from typical rodent research examining the acquisition and extinction of fear is that it fails to account for variable responses to aversive experiences. That is, the majority of individuals that experience a traumatic event do not develop post-traumatic stress disorder (PTSD) (Yehuda, 2004). In order to address this, researchers have developed animal models in which fear responses are exacerbated and/or extinction is impaired. For example, subjecting rats to an aversive experience before fear conditioning and extinction has been shown to result in deficits in extinction and is considered an animal model of PTSD (see Holmes and Wellman, 2009; Siegmund and Wotjak, 2007 for reviews). Specifically, a single prolonged stress treatment consisting of a combination of restraint stress, a forced swim test and injection of an anaesthetic drug has been found to have no effect on contextual fear conditioning but has been found to impair extinction relative to control rats (Yamamoto *et al.*, 2008). Similar results have been reported from studies using forced swimming in mice (Izquierdo *et al.*, 2006), restraint stress in rats (Miracle *et al.*, 2006) and elevated platform stress in rats (Akirav *et al.*, 2009). Research is underway to explore the neural and molecular signatures of these PTSD models with a goal to refine pharmacological adjuncts for PTSD in humans (e.g. Ponomarev *et al.*, 2010). Another approach to examining the development of PTSD in the laboratory has been exploration of the neuroscience of individual differences in fear acquisition and extinction. For example, genetic strains of rodents that are selectively bred to exhibit high or low anxious behaviour have also been shown to exhibit impaired extinction, despite there being no difference in the levels of fear acquisition in the different strains, an effect that has been demonstrated in both rats (Muigg *et al.*, 2008) and mice (Hefner *et al.*, 2008). As another example, LeDoux and colleagues examined fear reactivity and recovery in standard lab rats and established reliable phenotypes for rats that were more likely to acquire high levels of fear and rats that were slower to extinguish fear responses (Bush *et al.*, 2007). They concluded that high fear reactivity or slow recovery might be predictors of the development of PTSD. Indeed, research has shown that poor performance on a fear extinction task before trauma exposure is associated with an increased risk of developing PTSD (Guthrie and Bryant, 2006). Collectively, these approaches may reflect more ecologically valid models of the aetiology of anxiety disorders than standard fear conditioning methods, although there is no doubt that the study of extinction even in standard rodents has contributed substantially to our knowledge regarding the neural and molecular substrates underlying fear inhibition (see 'clinical utility of models of fear reduction' below).

US habituation

While extinction is widely considered to be the most valid model of exposure therapy, it is clear that extinction models only one aspect of exposure therapy: correction of irrational beliefs regarding the probability of negative events occurring. Extinction does not model the other important aspect of exposure therapy that involves the correction of irrational beliefs about the cost of the perceived negative outcome. In contrast, the seldom used 'US habituation' protocol does model this aspect of exposure therapy. In this procedure an animal is conditioned to fear a CS by pairing it with an aversive US, and the US is subsequently repeatedly presented by itself. This procedure reduces conditioned responding to the CS even though the CS was never extinguished. The reduction in responding to the CS is thought to be due to the US representation having been devalued as a result of the repeated exposure to the US (Rescorla, 1973). In other words, US habituation reduces the perceived cost of the negative outcome. US habituation is frequently used in clinical settings as a critical aspect of exposure therapy. For example, an individual with social phobia may be encouraged to deliberately draw attention to themselves by wearing an outlandish outfit in order to learn that the feared outcome, or US (e.g. people staring and laughing), is not as catastrophic as originally thought. This is different to the extinction aspect of exposure therapy, which teaches the individual that the feared outcome is unlikely to happen. Both CS extinction and US habituation result in the same behavioural outcome (i.e. reduced responding to a feared CS), although via different psychological mechanisms (i.e. extinction reduces the predictive value of the CS and habituation reduces the affective value of the US). Common treatments for anxiety disorders involve both habituation and extinction-like procedures and the patient learns that the feared outcome is unlikely to occur, and even if it does, it will not be catastrophic. Despite

their differences, recent research suggests that CS extinction and US habituation share some characteristics. Specifically, US habituation is context-dependent (i.e. fear responses are renewed when the CS is tested in a different context to that in which the US was habituated) and fear responses are reinstated when animals are exposed to an unexpected stressor before test (Storsve *et al.*, 2010). Furthermore, like CS extinction (Falls *et al.*, 1992) US habituation also depends on the activation of the N-methyl-D-aspartic acid (NMDA) receptor. Specifically, rats show high levels of freezing when tested with the CS following US habituation that was preceded by administration of an NMDA antagonist, suggesting that NMDA antagonism impairs US habituation (Storsve *et al.*, 2010). Therefore, while US habituation is rarely empirically studied, it seems that examining whether agents that enhance CS extinction also enhance US habituation would be beneficial and provide a more complete understanding of how to increase the efficacy of exposure therapy.

Memory reconsolidation

All new memories undergo a consolidation process, and interrupting this process prevents the transition from short-term to long-term memory (McGaugh, 2000). Reconsolidation is based on the idea that if an old memory is reactivated, by presenting a reminder of the original experience for example, it re-enters a labile state and must undergo a similar (re)consolidation process. Memory consolidation is mediated by a complex molecular process involving NMDA receptor activation, protein synthesis and gene transcription (Kandel, 2001). From this perspective, administration of agents that block components of this cascade should block consolidation and reconsolidation but have no effect on a stable, inactive long-term memory. Indeed, intra-amygdala infusions of anisomycin (a protein synthesis inhibitor) (Nader *et al.*, 2000) or systemic injections of an NMDA receptor antagonist (Lee *et al.*, 2006) were found to block reconsolidation of a reactivated fear conditioning memory; these treatments given when the memory had not been reactivated had no effect. Blocking reconsolidation results in low levels of fear when rats are subsequently tested for responding to the feared stimulus (see Nader and Einarsson, 2010, for a recent review on reconsolidation). Further, reactivation of the fear conditioning memory, by presenting a feared CS 10 min or 1 h before extinction training, has also been reported to attenuate reinstatement, renewal and spontaneous recovery of fear in rats (Monfils *et al.*, 2009). These results, coupled with research showing that the reconsolidation of aversive memories in humans can be disrupted (Holmes *et al.*, 2009; Kindt *et al.*, 2009; Schiller *et al.*, 2010), suggests that preventing the reconsolidation of fearful memories in humans with anxiety disorders may be an effective treatment strategy (see Ressler and Mayberg, 2007, for a discussion of this approach).

It should be noted that while disrupting reconsolidation produces the same behavioural outcome as CS extinction and US habituation, it is thought to do so by very different mechanisms. Specifically, extinction is thought to be mediated by the formation of a new memory (one in which the CS does not predict the US in the specific environment in which extinction occurred). Given that US habituation is also susceptible to renewal and reinstatement, it may be the case that US habituation is also mediated by the formation of a new memory (i.e. one in which the affective value of the US is reduced in the specific environment in which US habituation occurred). In contrast, procedures that disrupt reconsolidation are thought to be due to an erasure/modification of the original fear memory.

The clinical utility of models of fear reduction

A potential problem for research examining the effects of drugs on fear inhibition is that extinction, habituation and reconsolidation are all dependent on similar molecular signals (e.g. NMDA receptor activation, e.g. Falls *et al.*, 1992; Lee *et al.*, 2006; Storsve *et al.*, 2010). Therefore, drugs that enhance extinction and habituation might enhance, rather than disrupt, reconsolidation, which would lead to different behavioural outcomes depending on which process (i.e. extinction/habituation vs. reconsolidation) was dominant at the time of drug administration. If extinction/habituation is enhanced, then responding will decrease but if reconsolidation is enhanced, then responding will increase because the original fear memory has been strengthened. Likewise, drugs that impair reconsolidation (leading to decreased responding) may also impair extinction and habituation (leading to increased responding) depending on which process is dominant. In the laboratory it is relatively easy to control whether a memory is merely reactivated (and thus subject to reconsolidation) or extinguished by the length of the stimulus exposure, as a single short exposure is thought to result in memory reactivation while several exposures (or one long exposure) is thought to result in extinction. However, this would obviously be more difficult to control in real-world clinical settings and is therefore an issue that needs to be carefully considered in combining pharmacological adjuncts with exposure therapy.

Another important point is that extinction, habituation and reconsolidation might appear to be overly simplistic models of the treatment of anxiety disorders in humans. However, at least for the extinction model, this is not the case. Indeed, exposure therapy and extinction seem to be mediated by similar cognitive processes (see Hofmann, 2008, for recent review), and the pure clinical analogue of extinction (exposure therapy alone) is generally equally as effective in producing cognitive changes in threat expectancy as comprehensive cognitive-behavioural therapy (which incorporates exposure and cognitive restructuring; Hofmann, 2008). This suggests that exposure to feared stimuli without aversive outcomes (i.e. extinction) is potent enough to result in cognitive changes. Therefore, it is not surprising that research on extinction in rodents has proven to have powerful translational value (however, it should be remembered that extinction does not model the cost devaluation aspect of exposure therapy, as noted in the 'US habituation' section). Some notable recent examples of the direct applicability of behavioural neuroscience to clinical populations include findings that the neural circuitry involved in extinction in rodents (reviewed in Quirk and Mueller, 2008) is very similar to the neural circuitry that is activated in humans undergoing extinction of learned fear, and that this system has been shown to be dysfunctional in people with PTSD (e.g. Milad *et al.*, 2009b; Shin and Liberzon, 2010). Indeed, the same neural circuitry that is dysfunctional in humans with PTSD is also dysfunctional in inbred strains of mice that exhibit

impaired extinction (Hefner *et al.*, 2008). Furthermore, research examining the neural correlates of successful pharmacotherapy or psychotherapy reveals that reductions in anxiety as a result of treatment are associated with modifications and in some cases even a reversal of the dysfunctional neural activation in response to feared stimuli in individuals with anxiety disorders (see Porto *et al.*, 2009, for a recent review). This translation of behavioural neuroscience research to clinical populations is encouraging and suggests that we should continue to endeavour to understand extinction in the rodent. The hope is that increased understanding of models of fear inhibition or reduction will assist in the development and enhancement of treatments for anxiety disorders. Along these lines, the next part of this review presents evidence concerning several key drugs that have been shown to enhance fear inhibition via the above described models. We first review drugs that are currently in clinical use (either in clinical drug trials or in commercial use), and then review drugs that are still in the preclinical stages of investigation (see Table 2 for a summary).

Pharmacological adjuncts to therapy for anxiety disorders in humans

D-cycloserine (DCS) and other glutamate-based drugs

D-cycloserine is a partial agonist of the glycine site of the NMDA receptor and has been shown to facilitate the extinction of Pavlovian fear conditioning in rodents. Specifically, rodents given DCS either before or following extinction training show lower levels of fear the following day compared with rats injected with saline (e.g. Walker *et al.*, 2002; Ledgerwood *et al.*, 2003; Woods and Bouton, 2006; Yamada *et al.*, 2009). This result has been shown when the drug is injected systemically or when directly infused into brain regions instrumental in extinction such as the amygdala, and is robust, with a recent meta-analysis indicating large effect sizes (overall $d = 1.19$) for animal studies examining DCS and fear extinction (Norberg *et al.*, 2008).

Preclinical studies have revealed a number of exciting features of DCS including that it attenuates relapse after extinction training and slows reacquisition (Ledgerwood *et al.*, 2004; Bertotto *et al.*, 2006; but see Woods and Bouton, 2006). DCS can also produce what has been termed 'generalized extinction' (Ledgerwood *et al.*, 2005), whereby rats treated with DCS following extinction of one CS exhibit reduced fear responses to another CS (that was paired with the same US) whereas saline-treated animals only show low levels of fear to the CS subjected to extinction training. Importantly, DCS appears to facilitate the learning occurring during extinction training as opposed to resulting in state reductions in anxiety (see Richardson *et al.*, 2004; Vervliet, 2008, for reviews).

Therefore, it is no surprise that a number of well-designed recent studies have shown that DCS is an efficacious pharmacological adjunct to exposure-based therapies for anxiety disorders in humans. DCS has been shown to facilitate virtual reality therapy for acrophobia (fear of heights) (Ressler *et al.*, 2004), and exposure therapy for social anxiety disorder (Hofmann *et al.*, 2006; Guastella *et al.*, 2008), obsessive compulsive disorder (Kushner *et al.*, 2007; Wilhelm *et al.*, 2008; but see Storch *et al.*, 2007), and panic disorder (Otto *et al.*, 2010b). In many of these studies the benefits of DCS were apparent after only a few exposure sessions, suggesting that DCS enhances therapeutic learning and may reduce the number of treatment sessions required for significant symptom reduction. The calculated effect sizes for DCS as an adjunct to exposure therapy in clinical populations is estimated to be moderate ($d = 0.60$), suggesting that there is good preliminary evidence that DCS may have some clinical utility as an adjunct to psychotherapy (Norberg *et al.*, 2008).

Although DCS is the flagship for the translational potential of animal models of extinction, there are a number of potential limitations of the drug that have been discovered in the laboratory. For example, DCS does not facilitate extinction when rats have been pre-exposed to the drug or to an antidepressant (Parnas *et al.*, 2005; Werner-Seidler and Richardson, 2007). Additionally, DCS may not facilitate extinction in infant rats, where extinction is NMDA-independent (Langton *et al.*, 2007); however, DCS does facilitate extinction in adolescent rats, which typically show poor long-term extinction retention (McCallum *et al.*, 2010). It is also the case that DCS may be ineffective if the CS had previously been extinguished, although this effect is stimulus-, context- and time-dependent (Langton and Richardson, 2008; 2009; 2010a). These latter findings may be due to re-extinction involving the retrieval of a previously consolidated extinction memory, a process that does not appear to depend on NMDA-receptor activation (see Langton and Richardson, 2008). Another potential limitation regarding the use of DCS in the treatment of anxiety disorders is that it has been shown to enhance reconsolidation (Lee *et al.*, 2006), and therefore may potentially result in an incubation of fear if a fearful memory is activated during exposure therapy without actually being extinguished.

Finally, research showing that presentations of multiple footshocks before DCS administration can prevent it from facilitating extinction (Langton and Richardson, 2010b) and that DCS may produce anxiogenic effects in high anxiety (but not low anxiety) rats (Wu *et al.*, 2008) suggests that DCS may have different effects on behaviour depending on recent aversive experiences or basal levels of anxiety. Clinically, this may suggest that caution should be taken when delivering DCS soon after traumatic events.

However, it should be noted that research to date has shown that while within-session extinction learning is required in order for DCS to facilitate extinction, DCS administration does not result in fear incubation in the absence of a significant reduction in fear (Weber *et al.*, 2007; Bouton *et al.*, 2008; Hefner *et al.*, 2008). Second, compared with the large body of research showing DCS enhancement effects on extinction (see Norberg *et al.*, 2008, for a meta-analysis) there are only a handful of studies showing that DCS enhances CS or contextual fear conditioning (Silvestri and Root, 2008; Kalisch *et al.*, 2009; Waddell *et al.*, 2010; but see Yamamoto *et al.*, 2008). The fact that DCS does not generally enhance fear conditioning or produce fear incubation means that administration of DCS in combination with exposure therapy that fails to produce learning (i.e. does not produce reductions in anxiety) should not have harmful consequences

Table 2

Summary of research on pharmacological adjuncts to therapy for anxiety disorders in humans and drug targets currently being examined in preclinical animal models

		Effect on fear reduction in animal treatment models				Human studies	
Drug (s)	**Action**	**State/Trait anxiety**	**Disrupts reconsolidation**	**Extinction**	**US habituation**	**Safe for use**	**Trialled in anxiety disorders**
Drug targets currently being examined/used for anxiety disorders in humans							
Benzodiazepines	Increase GABAergic transmission	↓	✓	X	?	Y	Y
Cortisol (humans); corticosterone (rats)	Increase glucocorticoid levels	↑	?	✓	?	Y	Y
D-cycloserine (DCS)	NMDA partial agonist	—	X	✓	?	Y	Y
Propanolol	β-adrenergic antagonist	↓	✓	✓/X	?	Y	Y*
Selective serotonin reuptake inhibitors (SSRIs)	Increase levels of circulating serotonin	↑↓	?	?	?	Y	Y
Yohimbine	Noradrenergic antagonist	↑	?	✓~	?	Y	Y*
Drug targets currently being examined to reduce fear/anxiety in preclinical animal models							
Anisomycin	Inhibits protein synthesis involved in memory consolidation	?	✓	X	?	N	N
Brain-derived neurotrophic factor (BDNF)	Regulates synaptic plasticity and memory	?	—	✓	?	Y	N
Cannabinoid type 1 (CB1) agonist/endocannabinoid (eCB) uptake inhibitor	Increases endocannabinoid levels	↓ (low doses)	?	✓~	?	?	N
CB1 antagonists	Decreases endocannabinoid levels	↑	✓	X	?	?	N
Estrogen	Female sex hormone	↓	?	✓	?	Y	N
Fibroblast growth factor-2 (FGF2)	Enhances the molecular cascade involved in memory consolidation	↓	?	✓	?	Y	N
Histone deacetylase inhibitors	Increases histone acetylation	?	X	✓	?	Y	N
Methylene blue	General metabolic enhancer	↓	?	✓	?	Y	N

Please note the following nomenclature: ↓, reduction in fear; ↑, increase in fear; —, no effect; ?, unknown; ✓, enhances; X impairs; *, 1 study only; ~, 1 or more null or negative result; Y, yes; N, no.

(such as significant increases in levels of fear). It should be noted that with the exception of research showing that DCS can facilitate reconsolidation, none of the potential limitations of DCS are harmful, rather, use of DCS in a contraindicated scenario such as during re-extinction or in individuals also taking antidepressants merely produces a null effect.

Finally, it may be the case that DCS will also enhance US habituation given that an NMDA receptor antagonist impairs US habituation (Storsve *et al.*, 2010); however, this possibility is yet to be examined in the laboratory or in clinical trials. In order to improve our current understanding of the translational potential of DCS, both large-scale randomized controlled trials and applied research to examine the effects of DCS in different populations including community samples as well as adolescents and children are required. In this regard, a preliminary study examining the effect of combining DCS with cognitive behavioural therapy for obsessive compulsive disorder in 8–17 year olds found small to moderate treatment effects of the combined treatment (although the effect was not significant), suggesting that it would be worthwhile to conduct larger clinical trials in this age group (Storch *et al.*, 2010).

It should be noted that DCS is not the only glutamate-based drug that has been examined with respect to extinction. As just one example, the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor agonist 4-[2-(phenylsulfonylamino)ethylthio]-2,6-difluorophenoxyacetamide (PEPA) has been shown to facilitate extinction of fear in mice when administered systemically and when infused directly into the medial prefrontal cortex or the basolateral amygdala (Zushida *et al.*, 2007; Yamada *et al.*, 2009). Like the NMDA receptor, the AMPA receptor is a glutamate ionotropic receptor that is involved in learning and memory, including extinction. For a more detailed review of the role of glutamate receptors in fear extinction, including a review of other glutamate-based drugs that have been shown to enhance extinction, see Myers *et al.* (2011).

Propranolol

Propranolol is a beta-adrenergic receptor antagonist that has been shown to reduce state levels of anxiety in rats (as measured by the open field, Angrini *et al.*, 1998; and by light-potentiated startle, Walker and Davis, 2002) and in humans (as measured by levels of performance anxiety during a public speech, Hartley *et al.*, 1983). In addition, propranolol has also been shown to regulate the storage of emotional memories with systemic administration of propranolol consistently being shown to block the consolidation of emotional memories when administered at the time of initial training in rats and humans (Dalmaz *et al.*, 1993; Cahill *et al.*, 1994). In addition, animal research has shown that propranolol also blocks the reconsolidation of reactivated fear memories when administered immediately following the reminder cue (e.g. a single CS presentation; Dębiec and LeDoux, 2004).

Research examining the effect of propranolol on consolidation and reconsolidation of learned fear suggested that it might be used in clinical settings in two ways: first, it is possible that administration of propranolol immediately after a traumatic event might prevent the consolidation of the memory of the event and so prevent subsequent development of PTSD. This possibility was examined by Pitman *et al.* (2002), who recruited Emergency Department trauma-exposed patients and compared the effects of 10 days of propranolol (the first administration was received within 6 h of the trauma) versus a placebo on subsequent development of PTSD symptoms. Although most patients' symptoms of PTSD had reduced to subclinical levels at 3 months post-treatment, propranolol-treated patients exhibited significantly less physiological responding during script-driven imagery of the traumatic event 3 months post-treatment compared with placebo-treated patients. A subsequent study (Vaiva *et al.*, 2003) demonstrated that PTSD symptoms 3 months post-trauma were lower in patients who agreed to 7 days of propranolol treatment (three times daily, followed by a taper period of 8–12 days) compared with those who refused propranolol treatment but agreed to participate in the study. These studies provide preliminary evidence that immediate post-trauma treatment with propranolol may prevent the development of PTSD (although see Stein *et al.*, 2007).

While the premise that propranolol may be used to prevent the development of PTSD is attractive, it also must be acknowledged that in many cases it is not feasible to expect that propranolol can be administered immediately after the traumatic experience (as just one example, victims of sexual assault may not reveal their experiences until many years after the event, by which time the memory is stable). However, the preclinical study by Dębiec and LeDoux (2004) demonstrated that propranolol can disrupt a previously consolidated memory that has been reactivated, and presumably rendered labile. This suggests that another possible use for propranolol in clinical settings is to disrupt the reconsolidation of traumatic memories that are recalled during therapy. Preliminary support for this premise was provided by Brunet *et al.* (2008), who gave PTSD patients a single dose of propranolol or placebo immediately after they recalled their traumatic experience. Upon subsequent recall of the trauma 1 week later, propranolol-treated patients showed significantly reduced levels of physiological responding compared with placebo-treated patients.

Given that propranolol disrupts the consolidation of newly formed memories, one potential concern regarding the use of propranolol as an adjunct to treatment for anxiety disorders is that it may disrupt the consolidation of new extinction memories. Studies with rodents on this issue have produced mixed results, with some reporting that intra-infralimbic cortex (Mueller *et al.*, 2008) or intra-basolateral amygdala (Berlau and McGaugh, 2006) infusions of propranolol impair long-term extinction while others have reported no effect of systemic administration of propranolol on long-term extinction (Cain *et al.*, 2004; Rodriguez-Romaguera *et al.*, 2009). The differences between these studies may be due to mode of administration (i.e. central vs. peripheral) or due to parametric variance (i.e. in the length and amount of extinction training, resulting in differences regarding whether reconsolidation or extinction processes are dominant). In humans, it has been reported that patients who received propranolol before exposure therapy for agoraphobia showed significantly worse long-term extinction (indexed by spending less time travelling alone in the month following exposure therapy) compared with patients who received a placebo (Hafner and Milton, 1977). It may be the case that

propranolol is not suitable as an adjunct to treatment for anxiety disorders other than PTSD. In any case, it is clear that more research needs to be conducted to determine the effect of propranolol on reconsolidation versus extinction of the original fear. Furthermore, very little research has examined the effect of propranolol on US habituation. One study has shown that propranolol impairs long-term habituation of the acoustic startle response (Leaton and Cassella, 1984); however, to our knowledge no one has assessed the effects of combined propranolol and US habituation on subsequent learned or unlearned fear responding.

Benzodiazepines

Benzodiazepines, which bind to and increase the activity of the gamma-amino-butryric-acid receptor, are among the most widely prescribed drugs for the treatment of anxiety disorders. Benzodiazepines reduce state and trait levels of anxiety; their efficacy in this regard has been well-established in animal and human studies that have mainly utilized fear-potentiated startle as a measure (reviewed in Davis *et al.*, 1993).

It should be noted that while benzodiazepines reduce the symptoms of anxiety, they do not target the cognitive errors regarding beliefs about the probability and cost of negative events that are believed to maintain anxiety disorders. Furthermore, treatment with benzodiazepines alone is associated with high relapse rates upon discontinuation of the drug (Marks *et al.*, 1993; Spiegel *et al.*, 1994). However, it may be argued that benzodiazepines reduce state levels of anxiety to a point where an individual may feel confident enough to participate in exposure-based therapies. Indeed, many individuals with anxiety disorders undergo combined treatment with exposure therapy and benzodiazepines. Therefore it must be asked, what are the effects of benzodiazepines on extinction, habituation and reconsolidation? Animal research has consistently demonstrated that administration of benzodiazepines, either before or immediately after extinction, impairs long-term memory for extinction (Pereira *et al.*, 1989; Bouton *et al.*, 1990; Hart *et al.*, 2009), suggesting that benzodiazepines may actually reduce the beneficial effects of exposure therapy in clinical practice. Otto *et al.* (2010a) have suggested that concurrent use of benzodiazepines with exposure therapy may interfere with the consolidation of the extinction memory; however, there have been few controlled studies investigating the effects of combined treatment with benzodiazepines and exposure therapy in humans. One large-scale study (Marks *et al.*, 1993) compared the effectiveness of 8 weeks of the benzodiazepine alprazolam alone, exposure therapy alone, and combined alprazolam and exposure therapy in 154 patients with Panic Disorder. While both alprazolam alone and exposure alone led to improvements on outcome measures at 43 weeks post-treatment, the effect size for exposure alone was twice that of alprazolam alone. Further, the combination of alprazolam and exposure therapy was not superior to either drug or therapy alone, rather, the addition of alprazolam to exposure therapy actually prevented further treatment gains seen after the 8 week active treatment period in the exposure-alone group. Subsequent studies examining a variety of anxiety disorders have suggested that combination treatment at best leads to no better outcomes than either treatment alone, and at worst it impairs the beneficial effects of exposure therapy (reviewed in Pontoski and Heimberg, 2010). In light of these findings, it will be interesting for future research to assess the effect of benzodiazepines on long-term habituation. While studies have investigated the effects of benzodiazepines on the habituation of the startle response (e.g. Young *et al.*, 1991), to our knowledge no studies have examined the effects of benzodiazepines on long-term habituation when subjects are tested drug free. This is important given that benzodiazepines may reduce the expression of the startle response but not have any effect on the learning processes involved in habituation. Because preliminary data suggest that US habituation and CS extinction rely on similar neural mechanisms (Storsve *et al.*, 2010) it is possible that benzodiazepines may also impair long-term habituation.

Based on suggestions that benzodiazepines may interfere with memory consolidation (e.g. Otto *et al.*, 2010a) this class of drugs may be useful in disrupting the reconsolidation of traumatic memories. Indeed, there have been two studies that have examined the effects of benzodiazepines on the reconsolidation of reactivated learned fear. Both of these studies used rats as subjects, and both indicated that post-reactivation administration of benzodiazepines disrupted the reconsolidation of learned fear (Bustos *et al.*, 2006; Zhang and Cranney, 2008). These findings have yet to be explored in humans; however, they suggest that benzodiazepines, like propranolol, may be useful in disrupting the reconsolidation of traumatic memories in clinical settings (see Makkar *et al.*, 2010, for a review).

Selective serotonin reuptake inhibitors (SSRIs)

In the past two decades, SSRIs have overtaken benzodiazepines as the first-line pharmacological treatment for anxiety disorders. SSRIs prevent the reuptake of serotonin by the serotonergic neuron, thus increasing the availability of serotonin in the brain. SSRIs appear to be anxiolytic in humans, although paradoxically, they appear to be anxiogenic in preclinical rodent models of anxiety (reviewed in Kent *et al.*, 1998). Nevertheless, in general, treatment with SSRIs in humans has proven to be as effective as cognitive-behavioural therapy in a number of studies across a variety of anxiety disorders, although like benzodiazepines, relapse following medication discontinuation is a common problem (see Otto *et al.*, 2004, for a review).

Despite their pervasive use in clinical settings, very few preclinical studies have examined the effects of SSRIs on fear extinction in rodents. One study found that 4 weeks of fluoxetine treatment had no effect on fear extinction recall in two strains of mice differing in anxiety behaviour (Norcross *et al.*, 2008). This is consistent with the large body of research that has examined the effect of combining exposure therapy with SSRI treatment. The majority of this work has found no advantage of combined treatment over either treatment alone. For example, Simpson *et al.* (2004) found that patients with Obsessive Compulsive Disorder who received exposure therapy with or without clomipramine showed reduced relapse 12 weeks after treatment in comparison with patients who received clomipramine alone; however, there was no difference in treatment response or relapse rates between patients treated with combined treatment versus those treated with exposure therapy alone. As another example,

combining exposure therapy for social anxiety disorder with fluoxetine has been found to be no more effective than either treatment alone as measured at the end of treatment (Davidson *et al.*, 2004) and one study found that combined treatment was associated with a deterioration of treatment gains measured 28 weeks after treatment termination (Haug *et al.*, 2003). Otto *et al.* (2010a) have suggested that medication such as SSRIs may have both impairing as well as facilitative effects on exposure therapy, hence the general finding of no benefit to combined medication and psychotherapy treatments. Future research is required to further elucidate the effect of SSRIs on extinction, as well as to determine the effect of SSRIs on reconsolidation and US habituation.

Noradrenergic drugs (yohimbine)

Yohimbine, a drug that acts on the noradrenergic system, is another drug that has been shown to facilitate extinction in animals. For example, administration of yohimbine before extinction training has been shown to result in lower levels of fear the following day in comparison with control animals (e.g. Cain *et al.*, 2004; Morris and Bouton, 2007; see Holmes and Quirk, 2010, for review). This effect has also been demonstrated in strains of mice that exhibit impaired extinction (Hefner *et al.*, 2008), although one study reported no effect of yohimbine on fear extinction as measured by conditioned freezing and suppression (Mueller *et al.*, 2009) and another study reported that while yohimbine enhanced extinction, it did not reduce renewal of fear (Morris and Bouton, 2007). Nevertheless, positive results have been seen in a clinical sample of claustrophobic individuals, with administration of yohimbine before two exposure sessions resulting in a significantly greater improvement in symptoms relative to placebo (Powers *et al.*, 2009). Yohimbine is anxiogenic in rats and in humans (see Holmes and Quirk, 2010), and as such, yohimbine may work in part by increasing the predictive error during extinction, thus accelerating extinction learning.

Glucocorticoids

Glucocorticoids are a class of stress hormone that may potentially augment exposure therapy in humans with anxiety disorders. The approach of using stress hormones to facilitate extinction is also grounded in rodent research showing that glucocorticoid agonists can facilitate extinction (see Bentz *et al.*, 2010, for review). The first demonstration of this came from Barrett and Gonzalez-Lima (2004) who demonstrated that systemic metyrapone (a corticosterone inhibitor) impaired long-term extinction of a tone CS in mice when administered 90 min before extinction training, while having no effect on within-session extinction. A more recent study replicated this result using intra-amygdala infusions of the glucocorticoid antagonist mifepristone, and also demonstrated that systemic injections of the glucocorticoid agonist dexamethasone facilitated extinction (Yang *et al.*, 2006). Systemic glucocorticoid administration has been shown to increase state anxiety in rats (Mitra and Sapolsky, 2008), and so like yohimbine, glucocorticoids may also increase the predictive error experienced by the rodent during extinction training. It should be noted that the latter study in rats failed to find an effect of acute or chronic glucocorticoid administration on fear extinction; however, in that study behavioural procedures occurred 12 days after acute injections and 2 days after termination of chronic injections; hence, it is likely that glucocorticoid administration needs to take place at the time of extinction training to effectively enhance extinction retention. In humans, a recent double-blind study showed positive effects of cortisone relative to placebo in combination with exposure to feared stimuli in social phobia and arachnophobia (Soravia *et al.*, 2006).

New drug targets currently being examined in animal models

There are a number of other drugs that have been successful in facilitating the reduction of fear in preclinical studies. Many of these are known to be safe for use in humans and are therefore potential therapeutic targets for the future.

Fibroblast growth factor-2 (FGF2)

Fibroblast growth factor-2 is a potent mitogen that is involved in the molecular cascade underlying long-term memory (for more detail, see Graham and Richardson, 2009a; 2010a). Systemic administration of FGF2 either before or immediately following extinction training has been shown to enhance long-term retention of extinction in juvenile rats, and to reduce susceptibility to reinstatement and renewal (Graham and Richardson, 2009b; 2010b). Systemic administration of FGF2 has been shown to rapidly cross the blood brain barrier (Cuevas *et al.*, 1996; Deguchi *et al.*, 2000) and so it is likely that FGF2 enhances extinction via central effects (see Perez *et al.* 2009 for a discussion on how FGF2 may alter blood–brain barrier permeability). However, it is also possible that FGF2 enhances extinction, in part, via peripheral effects. Future research that compares the effects of centrally versus systemically administered FGF2 on extinction is required to address this issue.

Interestingly, when FGF2 is administered before extinction training it reduces the level of freezing throughout the extinction training session (Graham and Richardson, 2009b). This converges with evidence suggesting that FGF2 may be a novel anxiolytic. For example, Perez *et al.* (2009) bred rats that exhibit either high or low trait levels of anxiety (as measured by responses to novelty and anxiety-provoking situations, including the elevated plus maze), and found that highly anxious rats had lower levels of hippocampal FGF2 messenger RNA (mRNA) expression compared with low-anxious rats. They also demonstrated that placement in an enriched environment (a situation known to reduce anxious behaviour; Benaroya-Milshtein *et al.*, 2004) reduced anxious behaviour in highly anxious rats and led to an increase of FGF2 expression in the hippocampus. Finally, they demonstrated that 3 weeks of treatment with exogenous FGF2 increased hippocampal neurogenesis and reduced anxious behaviour, an effect that was particularly pronounced in the highly anxious rats. The notion that acute FGF2 may have anxiolytic properties is also consistent with the finding that acute administration of diazepam, a commonly prescribed anxiolytic, leads to increases in FGF2 mRNA in the hippocampus and striatum in adult rats (Gomez-Pinilla *et al.*, 2000).

It is possible that increases in FGF2 activity in certain neural regions (e.g. the hippocampus) may be the mechanism of action for common anxiolytics; however, as rats were killed 6 h after diazepam administration, it is unknown whether the increase in FGF2 mRNA observed by Gomez-Pinilla *et al.* coincides with the time point at which benzodiazepines become therapeutically effective (i.e. almost immediately). Together, this research suggests that in addition to modulating extinction, FGF2 may also modulate state/trait levels of anxiety. Importantly, the effects of FGF2 on extinction cannot be due to the potential effects of FGF2 on state anxiety because FGF2 does not facilitate extinction when administered 4 h after extinction, or when no short-term extinction occurs (Graham and Richardson, 2009b). This demonstrates that FGF2 must be present during extinction in order to facilitate long-term extinction retention, suggesting that FGF2 is modulating some aspect of the extinction process independently of its effects on state anxiety (i.e. possibly by enhancing the consolidation of the extinction memory). The effects of FGF2 on US habituation and reconsolidation are yet to be examined; however, given that FGF2 is critical to the molecular formation of long-term memories, it is likely that FGF2 will enhance both US habitation and reconsolidation, and so again, combining FGF2 with extinction/habituation procedures may result in reduced or enhanced conditioned responding depending on which process is dominant (i.e. extinction/habituation or reconsolidation).

While FGF2 has not yet been trialled as an enhancer of extinction in humans, its safety for potential use has been demonstrated in several human clinical trials investigating the effects of FGF2 on angiogenesis (Laham *et al.*, 2000; Lazarous *et al.*, 2000; Lederman *et al.*, 2002). Together, these studies have indicated that acute administration of a range of doses of FGF2 is well-tolerated in humans.

Brain-derived neurotrophic factor (BDNF)

Emerging research has implicated BDNF as a critical signal in regulating synaptic plasticity and memory (see Lu *et al.*, 2008, for a review). In addition, preclinical studies in rodents have suggested that BDNF may be involved in the regulation of fear extinction. Elevated BDNF mRNA has been reported to occur in the basolateral complex of the amygdala following extinction of conditioned fear, and disruption of BDNF signalling via lentiviral infection disrupts long-term retention of extinction, without impairing within-session extinction (Chhatwal *et al.*, 2006). Very recently, it has been demonstrated that intra-infralimbic infusions of BDNF reduced expression of conditioned fear the day after infusions, and this effect occurred even in the absence of extinction training (Peters *et al.*, 2010). This suggests that BDNF does not reduce conditioned responding via modulating extinction-like processes, despite findings that BDNF may be involved in extinction regulation (Chhatwal *et al.*, 2006). Clearly more research is required to elucidate the role of BDNF in fear regulation. One possibility is that BDNF alters the original fear memory, a process that does not depend on extinction training; however, this is inconsistent with other findings showing that BDNF is not involved in the reconsolidation of fear memories, despite being involved in the initial consolidation of fear memories (Lee *et al.*, 2004). The safety of BDNF administration in humans has not yet been assessed.

Histone deacetylase inhibitors

DNA is packaged in proteins called 'histones', which tightly bind to DNA and prevent gene transcription when they are in an unmodified state. When histones become acetylated this neutralizes their positive charge and allows for gene transcription and expression as it disrupts the interaction between the positively charged histones and the negatively charged DNA (Levenson and Sweatt, 2005). Emerging evidence strongly implicates histone modification as one of the critical structural changes that occurs in the brain that regulates the persistence of long-term memories, including extinction memories. For instance, histone acetylation increases in the prefrontal cortex following extinction training (Bredy *et al.*, 2007). This raises the possibility that increasing histone acetylation may enhance extinction memories. Indeed, it has recently been demonstrated that systemic or intrahippocampal administration of histone deacetylase (HDAC) inhibitors (which increase histone acetylation) facilitate long-term memory for fear extinction in rodents (Bredy *et al.*, 2007; Lattal *et al.*, 2007; Bredy and Barad, 2008). This effect has been demonstrated when using a partial extinction protocol that typically leads to no long-term extinction retention, suggesting that HDAC inhibitors may enhance extinction under conditions in which it is normally impaired (Bredy and Barad, 2008). To our knowledge, the role of HDACs in US habituation has not yet been examined preclinically or clinically.

One disadvantage to the use of HDACs as potential pharmacological adjuncts to exposure-based therapy is that one study has shown that the HDAC Valproic Acid led to increased susceptibility to relapse following extinction in mice when using a within-subjects design (i.e. they exhibited greater renewal of fear when tested outside of the extinction context; Bredy and Barad, 2008). Furthermore, that study also demonstrated that Valproic Acid facilitated reconsolidation of conditioned fear, suggesting that HDACs may potentially incubate anxiety if combined with exposure therapy that does not produce sufficient fear extinction, a possibility that is yet to be examined. However, one advantage of using HDACs as a pharmacological adjunct to exposure-based therapy is that some, including Valproic Acid, are already being used as treatments in psychiatric settings as a mood stabilizer (Peterson and Naunton, 2005), and as such they can be immediately trialled in humans as an enhancer of extinction.

Estrogen

The female sex hormone estrogen plays a key role in modulating state levels of anxiety (Lund *et al.*, 2005), and has been shown to be involved in regulating the function and morphology of the neural circuitry underlying extinction learning (Goldstein *et al.*, 2001; Shansky *et al.*, 2010). Further, recent studies have demonstrated that systemic administration of estrogen improves the consolidation of fear extinction in female adult rats (Chang *et al.*, 2009; Milad *et al.*, 2009a). The potential of estrogen as an enhancer of extinction has not yet been examined in male rodents or in human clinical

samples; however, these preliminary studies suggest that estrogen manipulation may be a useful means of modulating extinction. These findings are particularly relevant given that women are twice as likely to develop anxiety disorders compared with men (Kessler *et al.*, 1994). Furthermore, estrogen is approved for use in humans by the food and drug administration, which may accelerate the emergence of clinical trials investigating the effect of estrogen on exposure therapy in humans.

Methylene blue

Methylene blue (MB) is a general metabolic enhancer that, when injected systemically, increases energy metabolism in the brain and has anxiolytic effects in rats (Eroglu and Çaglayan, 1997). MB has also been shown to enhance long-term extinction memory retention (Gonzalez-Lima and Bruchey, 2004; Wrubel *et al.*, 2007). MB enhances energy production throughout the entire brain, particularly in the prefrontal cortex, which is important for extinction consolidation and retention (Quirk and Mueller, 2008). Furthermore, MB crosses the blood–brain barrier and reaches a concentration 10–20 times greater in the brain compared with the blood 1 h after systemic administration (Peter *et al.*, 2000), suggesting that MB may act centrally, and in particular, on the prefrontal cortex, to enhance extinction. However, studies comparing the effects of centrally versus systemically administered MB on extinction will be needed to determine this. As MB is already approved for use by the food and drug administration, this drug may be an effective pharmacological adjunct to exposure therapy in individuals with anxiety disorders (Gonzalez-Lima and Bruchey, 2004).

Cannabinoids

The endocannabinoid (eCB) neurotransmitter system has been implicated in the inhibition of fear (e.g. Marsicano *et al.*, 2002). The importance of the eCB system, in particular the cannabinoid receptor type 1 (CB1) cannabinoid receptor, in extinction has been demonstrated using knockout mice (e.g. Marsicano *et al.*, 2002; Kamprath *et al.*, 2006) and systemic administration of eCB modulating agents (e.g. Chhatwal *et al.*, 2005; Pamplona *et al.*, 2006; 2008). Specifically, CB1 agonists such as WIN 55, 212-2 (e.g. Lin *et al.*, 2006; Pamplona *et al.*, 2006; 2008 but see Chhatwal *et al.*, 2005) and eCB metabolism uptake inhibitors such as N-(4-hydroxyphenyl)arachidonoylethanolamide (Chhatwal *et al.*, 2005; Pamplona *et al.*, 2008) have been shown to facilitate extinction. Interestingly, the mechanism through which the eCB system mediates extinction is thought to be via habituation processes (Kamprath and Wotjak, 2004; Kamprath *et al.*, 2006). Further, CB1 antagonists have been shown to block reconsolidation (Lin *et al.*, 2006). Taken together, this research suggests that drugs that target the eCB system may potentially enhance therapy in humans (see Chhatwal and Ressler, 2007, for review); however, government approval of drugs in the same family as cannabis is controversial and potentially risky given the association of these drugs with psychotic illness. Furthermore, given that CB1 antagonists block reconsolidation it is possible that CB1 agonists may incubate anxiety by enhancing the reconsolidation of reactivated fear memories. Finally, one study has demonstrated that chronic exposure to a CB1 receptor agonist impaired fear extinction, and the facilitating effect of a CB1 receptor agonist on extinction was significantly reduced in rats that were previously chronically exposed to the CB1 receptor agonist (Lin *et al.*, 2008). This suggests that CB1 agonists may not be effective if taken repeatedly, or in populations that have chronic prior exposure to CB1 drugs (e.g. cannabis users).

Summary and future directions

Animal models of exposure therapy have proved to be hugely effective in identifying the neural and molecular substrates underlying fear inhibition, and they have led to the identification of numerous drugs that enhance these processes in both rodents and humans. Fear extinction has been the most influential animal model of exposure therapy; however, it is important to note that fear extinction does not model the cost reappraisal aspect of exposure therapy, nor does it model reconsolidation of the original fear memory. This latter shortcoming has recently been acknowledged and more research is being done to examine the role of reconsolidation in fear inhibition and how disruption of reconsolidation can be modulated pharmacologically to achieve fear reduction. However, very little research to date has examined US habituation, which may prove to be a useful model of the cost reappraisal component of exposure therapy. It is clear that future research will benefit from examining the effect of pharmacological enhancers of extinction on US habituation and memory reconsolidation in order to provide a more complete understanding of how these agents may affect exposure therapy. This is even more pertinent when it is considered that extinction/habituation and disruption of reconsolidation likely depend on opposing molecular processes, such that administration of the same drug may lead to very different therapeutic outcomes depending on which process (i.e. extinction/habituation or reconsolidation) it enhances. Finally, a fundamental perspective underlying recent research on the pharmacological enhancement of extinction/exposure therapy is that it is critical to focus on the processes underlying the inhibition of the fear rather than the reduction in anxiety *per se* (Davis *et al.*, 2006). This approach is likely to lead to continued advancements in this important area of research.

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Conflict of interest

None of the authors have any conflict of interests to declare.

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