D-Cycloserine Facilitates Extinction of Learned Fear: Effects on Reacquisition and Generalized Extinction

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Background: *D-cycloserine (DCS) facilitates extinction of learned fear. The aim of this study was to examine whether DCS 1) affects reacquisition of fear (Experiment 1) and 2) produces generalized extinction of fear (Experiment 2).*

Methods: *Following fear conditioning, where a light or a tone conditioned stimulus (CS) was paired with a white-noise burst unconditioned stimulus (US), rats received nonreinforced exposure to one CS (i.e., extinction training). Fear was assessed by measuring CS-elicited freezing, a species-specific defense response.*

Results: *Rats given DCS exhibited facilitated extinction of fear but were able to reacquire fear of that CS in a similar manner as saline-treated control animals (Experiment 1). Furthermore, DCS-treated rats exhibited generalized extinction (i.e., they were less fearful of a non-extinguished CS) in comparison to controls (Experiment 2).*

Conclusions: *DCS facilitates extinction of learned fear to the extinguished CS, but also appears to reduce fear of a nonextinguished CS. These findings suggest that this drug may have substantial clinical value in the treatment of anxiety disorders.*

Key Words: D-cycloserine, extinction, learned fear, N-methyl-D-aspartate, startle modification, anxiety

Anxiety disorders are a leading psychological problem in the industrialized world. Many current treatments for these disorders are based on the concept of "extinction" whereby the uncontrolled fear that underlies anxiety is reduced by repeatedly exposing clients to the eliciting stimulus in a controlled environment where no adverse consequences occur.

Although extinction has been studied extensively in the laboratory and would seem to be a relatively straightforward process, it has proven to be much more complex than it appears. Most of the laboratory-based studies on extinction of fear involve Pavlovian conditioning, a procedure in which an initially neutral stimulus (the conditioned stimulus; CS) such as a light or a tone is repeatedly paired with an aversive stimulus (the unconditioned stimulus; US) such as shock. Following this training, some animals receive multiple, non-reinforced presentations of the CS (i.e., extinction). These animals exhibit substantially less fear of the CS when it is subsequently presented at test compared with animals not receiving extinction. Vastly different accounts of this loss in fear have been offered, however. For example, it has been suggested that extinction of learned fear could be due to either the "unlearning" of the CS–US association (e.g., Rescorla and Wagner 1972); the encoding of a second, opposing memory of the CS (i.e., CS predicts no US vs. CS predicts US; Bouton 1991); or the devaluation of the US representation (e.g., Rescorla 1973; Rescorla and Heth 1975). Interestingly, from both of these latter perspectives, extinction does not involve a loss of the original CS–US association.

At a neural level, there is relatively little known about the extinction of learned fear, especially in comparison to what we know about the acquisition of learned fear. There is, however, substantial evidence that the N-methyl-D-aspartate (NMDA) system is involved in extinction. Most of this evidence comes from studies where NMDA antagonists have been shown to block extinction (for an extensive review, see Myers and Davis 2002). For example, Falls et al (1992) reported that infusion of D,L-2-amino-5-phosphonovaleric acid (AP5) into the basolateral nucleus of the amygdala before extinction training dose-dependently blocked extinction of conditioned fear, as measured by fear-potentiated startle. Studies using alternative NMDA antagonists and alternative fear responses have also provided evidence for the involvement of the NMDA system in extinction (Baker and Azorlosa 1996; Cox and Westbrook 1994; Santini et al 2001).

More recently, NMDA agonists have been shown to facilitate the extinction of learned fear. Specifically, Walker et al (2002) demonstrated enhanced extinction of fear, as measured by the fear-potentiated startle procedure, when D-cycloserine (DCS), a partial NMDA agonist, was administered systemically and also when infused into the basolateral nucleus of the amygdala before extinction training. Ledgerwood et al (2003) replicated this finding with a cue-conditioned freezing paradigm and also demonstrated that DCS enhanced extinction in a time-dependent fashion when administered after extinction training, implicating consolidation processes. Both studies illustrated that it is the combination of the extinction training session and DCS administration, and not DCS administration alone, that is critical in producing the subsequent response reduction. Such findings are important not only because of what they tell us about the neural bases of extinction, but also because of the promise they hold for developing effective pharmacologic interventions to assist in the treatment of fear and anxiety disorders such as phobia, posttraumatic stress disorder, obsessive–compulsive disorder, and panic disorder.

Given that current behavioral treatments for fear and anxiety disorders are explicitly based on the process of extinction, any pharmacological agent that enhances extinction might also enhance the effectiveness of these therapies. This prospect is supported by a recent study reported by Ressler et al (2004) in which it was reported that DCS (administered orally before each of two sessions of virtual reality therapy) produced a faster reduction of acrophobia (abnormal fear of heights) than that seen in patients exposed to "virtual" heights and administered a placebo. In addition, the DCS-treated patients (who were only given two therapy sessions) exhibited clinical improvements that were at least equal to, and in some cases better than, those exhibited by control patients given eight therapy sessions.

Given the potential value of DCS, both clinically and in terms of elucidating the mechanisms involved in extinction, it would seem prudent to explore more fully some of the consequences of using DCS to facilitate extinction of fear. For example, it would

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be helpful to know whether postextinction training administration of DCS affects 1) the extinction of learned fear of different sensory modalities of US (to date only the tactile US, foot shock, has been used; e.g., Ledgerwood et al 2003; Walker et al 2002), 2) the subsequent ability of the animal to acquire fears, and 3) responding to another fear-eliciting CS (i.e., do DCS-treated animals exhibit more generalized extinction?).

In this study, therefore, we continued to explore the facilitation of extinction of learned fear by DCS. Specifically, we examined whether 1) DCS-treated rats show facilitated extinction when an auditory US (i.e., white-noise burst) is used, 2) DCS-treated rats can reacquire conditioned fear following extinction training, and 3) DCS-facilitated extinction generalizes to a second CS previously paired with the aversive US but not extinguished. The results of this type of analysis should prove useful in determining both the process by which DCS facilitates extinction and the potential clinical value of this agent in treating fear and anxiety-related disorders.

Methods and Materials

Animals

Naïve adult male Sprague–Dawley rats weighing between 300 and 350 g (Gore Hill Research Laboratories, Sydney, Australia) were housed in groups of eight in a colony room maintained on a 12-hour light–dark cycle for at least 1 week before starting the experiments (Exp. 1, $N = 24$; and Exp. 2, $N = 40$).

Apparatus

Rats were preexposed, conditioned, extinction trained, and tested in one of four standard conditioning chambers (20 cm long × 12 cm wide × 12 cm high). Each chamber consisted of a Perspex ceiling, stainless steel rear wall, stainless steel mesh sides, and a hinged Perspex front door that locked magnetically. The floor consisted of stainless steel rods, 2 mm in diameter, spaced 13 mm apart (center to center). Each floor was located 8 cm above a stainless steel tray that served to collect boli and urine. The chambers were contained in pairs in two sound-attenuating wooden cabinets, and each chamber was separated from its partner by a solid timber partition within each cabinet. The chambers could be viewed through a Perspex window in the front door of each cabinet. To prevent the rats from being distracted by extraneous visual stimulation, each chamber was illuminated by a 15-W red light bulb, and the experimental room was also illuminated by red light. Before each session, two of the chambers were wiped with .5% acetic acid (in tap water) and the alternate two with 1.0% vanilla (in tap water). All programming, timing, and startle stimulus presentations were computer controlled.

In each conditioning chamber, there were two piezoelectric speakers (Tandy, Australia, Type 40-1370), mounted 6 cm from each of the two side walls. These speakers were simultaneously activated to produce the auditory cue used in this study. The auditory CS consisted of a 1.33-kHz, 70-dB tone (Brüel & Kjaer, Type 2235, A scale). A 240-volt, 25-watt white light globe mounted 11 cm from the rear of each chamber was used as the visual CS (intensity of the light CS was 16–17 lux). The US consisted of a 100-msec, 120-dB white-noise burst (Brüel & Kjaer, Type 2235, linear scale). During the experiment, the rats were observed and their behavior recorded with a video camera positioned in front of the chambers. Plastic boxes (60 cm long × 28 cm wide × 16 cm high) served to transport rats between their home cage and the experimental chambers.

Pharmacologic Treatment

D-cycloserine (Sigma-Aldrich, Australia) was freshly dissolved in saline (.9% wt/vol) and injected subcutaneously (SC) in a volume of 1.0 ml/kg and a dose of 15.0 mg/kg. Control animals were subcutaneously injected with saline in a volume of 1.0 ml/kg. The drug dose was chosen on the basis of the results of other behavioral studies (Land and Riccio 1999; Ledgerwood et al 2003; Pussinen et al 1997; Walker et al 2002), and estimates of brain concentration after systemic administration (Loscher et al 1994).

General Behavioral Procedures

Experimental procedures followed the ethical guidelines established by the American Psychological Association and were approved by the Animal Care and Ethics Committee of the University of New South Wales. Experiments were performed between 9 AM and 5 PM.

Experiment 1. Experiment 1 employed a three-group design (see Table 1). On day 1, preexposure, animals were placed into the conditioning chambers on four occasions for 15 min each. The sessions were given in sets of two separated by 15 min, with the sets separated by 2 hour. No stimuli were presented during these sessions. Animals were preexposed to the conditioning context to reduce the influence of contextual associations on cue-conditioned freezing (i.e., via latent inhibition of the context; Kiernan and Westbrook 1993).

On day 2, fear conditioning, animals were placed into the conditioning chambers for two sessions separated by 2 hours. Each session consisted of seven presentations of a 20-sec white light CS that coterminated with a burst of loud white noise on a 120-sec variable interval. The first CS was presented 2 min after placement in the apparatus. Each animal remained in the chamber for 10 sec following the final stimulus presentation before being returned to its home cage.

On day 3, extinction training, animals were placed into the conditioning chambers for 24 min. Two minutes after the animals were placed into the chambers, the CS was presented for 2 min. The CS was presented six times during the 24-min session with a 4-min (onset to onset) intertrial interval (similar to Ledgerwood et al 2003). No bursts of loud white noise were delivered during the extinction training session. Following extinction training, animals were injected SC with either DCS or saline.

Table 1. Summary of Design for Experiment 1

	Day							
	1 Exp	2 Cond	3 Extn	4 Test	5 Extn	6 Test	7 Cond	8 Test
Sal-1	—	L-US	L/Sal	L	L	L	—	—
Sal-2	—	L-US	L/Sal	L	L	—	L-US	L
DCS	—	L-US	L/DCS	L	—	—	L-US	L

—, context only. Sal, Saline; DCS, D-cycloserine; Exp, exposure; Cond, conditioning; Extn, extinction training; L, light CS; US, unconditioned stimulus (loud white-noise burst).

On day 4, retention test, animals were placed into the conditioning chambers for 10 min. The CS was presented for 30 sec on four occasions: 6, 7, 8, and 9 min after placement in the chamber (similar to Ledgerwood et al 2004).

On day 5, extinction training, saline-treated animals were given a second extinction training session identical to the session outlined on day 3. Animals in the DCS group did not receive extinction training on day 5 and were exposed to the conditioning chambers only, for a period of 24 min. Animals were not injected with either DCS or saline on this day.

On day 6, retention test, half of the saline-treated animals (i.e., saline-1 group) were placed into the conditioning chambers for 10 min. The CS was presented for 30 sec on four occasions: 6, 7, 8, and 9 min after placement in the chamber.

On day 7, fear conditioning, the remaining half of the saline-treated animals (i.e., saline-2 group), and animals in the DCS group were given a single fear conditioning session in the conditioning chambers. The session consisted of seven presentations of a 20-sec white light CS that coterminated with a burst of loud white noise on a 120-sec variable interval. The first CS was presented 2 min after placement in the apparatus. Each animal remained in the chamber for 10 sec following the final stimulus presentation before being returned to its home cage.

On day 8, retention test, animals in the saline-2 and DCS groups were placed into the conditioning chambers and tested in a manner identical to that outlined on day 4.

Experiment 2. Experiment 2 employed four groups in a two (extinction, no extinction) by two (saline, DCS) design (see Table 2). On day 1, preexposure, animals received preexposure to the conditioning chambers in a manner similar to that of Experiment 1.

On day 2, fear conditioning, animals were placed into the conditioning chambers for two sessions separated by 2 hours. Each session consisted of 14 presentations of a loud white noise burst on a 120-sec variable interval. A 20-sec white light CS coterminated with seven of the startle stimulus presentations, and a 20-sec tone CS coterminated with the remaining seven stimulus presentations. Light and tone CS presentations were intermixed. The first stimulus was presented 2 min after placement in the apparatus. Each animal remained in the chamber for 10 sec following the final stimulus presentation before being returned to its home cage.

On day 3, extinction training, two groups of animals were placed into the conditioning chambers for two sessions separated by 2 hours. Each light extinction session was the same as that in Experiment 1. Two groups of animals did not receive extinction training on day 3 and were transported to the laboratory and handled only. Following either the second extinction training session or handling, animals were injected SC with either DCS or saline.

Table 2. Summary of Design for Experiment 2

	Day			
	1 Exposure	2 Conditioning	3 Extinction	4 Test
Sal-E	—	L-US, T-US	L/Sal	L; T
DCS-E	—	L-US, T-US	L/DCS	L; T
Sal-NE	—	L-US, T-US	—/Sal	L; T
DCS-NE	—	L-US, T-US	—/DCS	L; T

—, context only. Sal, Saline; DCS, D-cycloserine; E, extinction condition; NE, no-extinction condition; L, light CS; T, tone CS; US, unconditioned stimulus (loud white-noise burst).

On day 4, all animals were placed into the conditioning chambers for two 10-min sessions (one in the morning and one in the afternoon). Each session consisted of four presentations of one of the CSs (i.e., light or tone). The CS was presented for 30 sec on four occasions: 6, 7, 8, and 9 min after placement in the chamber. Half of the rats in each group were tested with the light in the morning and then with the tone in the afternoon, and the remaining rats received the reverse order.

Scoring and Statistics

Each animal was scored for freezing during extinction training and the retention test. Freezing was scored as the absence of all movement except that related to respiration (Fanselow 1994). Freezing was rated with a time-sampling procedure in which every animal was observed once every 2 sec. A percentage score was calculated for the proportion of the total observation period. The animals' behavior was also video recorded, and later a trained observer blind to the experimental conditions scored a representative sample of test behavior. The correlation between the scorers' ratings on those samples was .96. Percent freezing data were analyzed by analysis of variance (ANOVA) or independent-samples t tests. A p value $< .05$ was considered statistically significant.

Results

Can DCS-Treated Rats Reacquire Learned Fear in a Manner Similar to Saline-Treated Rats?

Recent studies have demonstrated that DCS facilitates extinction of learned fear to a light CS that had previously been paired with a foot-shock US (Ledgerwood et al 2003; Walker et al 2002). In Experiment 1, we attempted to extend this research by 1) using a different US (i.e., an acoustic white noise stimulus) and 2) examining whether DCS-treated rats were permanently impaired in acquiring fear (which would not be an adaptive outcome). Analysis of the extinction training data yielded a significant linear trend indicating that freezing decreased across trials, $F(1,21) = 258.60$, $p < .001$ (overall mean percent freezing on first trial = 83.33%, on last trial = .30%). At test on day 4, across the 30-sec period before the initial light CS presentation, only minimal levels of freezing were exhibited (overall M = .60%, SD = 2.92). When the light CS was presented on day 4, DCS-treated rats ($n = 8$) exhibited less freezing than did saline-treated rats ($n = 16$), $t(22) = 3.89$, $p < .001$ (see Figure 1), thus extending previous findings (Ledgerwood et al 2003; Walker et al 2002) in which foot shock had been used as the US.

Following an additional extinction training session for all saline-treated rats on day 5, half of the saline-treated rats were tested for freezing on day 6. The level of freezing exhibited by this group was now equivalent to that of DCS-treated rats on day 4, $t(14) = .51$ (see Figure 1). On day 7, the DCS-treated rats and the remaining half of the saline-treated rats received a single session of conditioning. Figure 1 shows that at test on day 8, there was no difference between the saline and DCS groups, $t(14) = .12$. Thus, it appears that DCS facilitates extinction of conditioned fear to a CS without interfering with subsequent learning about that CS and the US.

Does DCS-Facilitation of Extinction Generalize to Another CS Previously Paired with the Aversive US?

In Experiment 2, we examined whether postextinction training administration of DCS affects responding to another CS that was previously paired with the aversive US but not extinguished (i.e., do DCS-treated rats exhibit generalized extinction?).

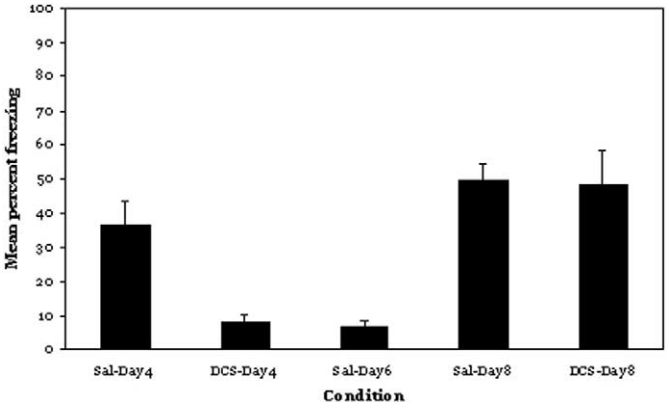

Figure 1. Effect of D-cycloserine (DCS) on conditioned freezing during a series of three tests in Experiment 1. Mean (+ SEM) percentage of time rats spent freezing during four 30-sec presentations of the light conditioned stimulus following a single extinction training session (day 4), a second extinction training session for the saline (Sal) group only (day 6), and fear reacquisition (day 8).

Analysis of the extinction training data for the two extinction groups ($n = 20$) yielded a significant linear trend, indicating that freezing decreased across trials, $F(1,18) = 119.83$, $p < .001$. A significant session-by-trial linear trend indicated that there was less freezing at the beginning of the second session compared with the beginning of the first session $F(1,18) = 68.27$, $p < .001$ (overall mean percent freezing session 1, trial 1 = 73.39%; session 1, trial 6 = .00%; session 2, trial 1 = 18.91%; session 2, trial 6 = .00%).

At test, only minimal levels of freezing were exhibited during the 30-sec period before the initial CS presentation (light CS: overall M = 2.14%, SD = 6.10; tone CS: overall M = .00%, SD = .00). Figure 2A (depicting performance on the light CS test) shows that 1) rats given extinction training displayed significantly less freezing to the light than rats not given extinction training, $F(1,36) = 19.29$, $p < .01$, and 2) DCS administration following extinction training led to a significantly greater reduction in freezing to the light than that observed following saline administration, $t(18) = 2.44$, $p = .026$. These findings replicate those reported in Experiment 1.

Of critical interest is the pattern of findings obtained with the tone CS, shown in Figure 2B. If DCS-treated rats exhibit generalized extinction, then conditioned freezing to the tone CS should also be reduced. Alternatively, if DCS-facilitation of extinction does not generalize to another CS that was previously paired with the aversive US, then conditioned freezing to the tone CS should not be affected. As Figure 2 suggests, and as demonstrated by a significant interaction, $F(1,36) = 16.12$, $p < .01$, the combination of extinction training to the light CS and DCS administration led to significantly reduced freezing to the tone compared with that seen in rats given saline after extinction of the light CS or those rats not given any extinction training. These results suggest that DCS-treated rats exhibit generalized extinction.

Discussion

This study replicates the previous finding that DCS facilitates extinction of learned fear (Ledgerwood et al 2003; Walker et al 2002). Because DCS was administered after extinction training, the processes involved in such facilitation are likely to be affecting consolidation rather than acquisition processes (see Santini et al 2001). Furthermore, this study extends that original finding to a different aversive US than that used previously (i.e., white-noise burst rather than shock). In addition, Experiment 1 also found that DCS administration following extinction training did not affect the animals' ability to subsequently reacquire fear memories about the original CS and US. In Experiment 2, it was found that administration of DCS leads to a generalization of extinction to another CS previously paired with the same US but not extinguished. This latter finding is of particular interest given that extinction of one CS does not usually lead to loss of fear to a second CS (an effect replicated in the saline-treated rats in Experiment 2).

One novel finding from this study is that DCS facilitates extinction of learned fear when a white-noise burst, instead of shock, is used as the US (Ledgerwood et al 2003; Walker et al 2002). At this point, it is unclear whether DCS facilitation of extinction is restricted to aversive conditioning because to our knowledge, only one study has examined the effects of this agent on the extinction of an appetitive CS. Port and Seybold (1998) demonstrated that preextinction training DCS administration prevented the extinction of an appetitive bar-press response, whereas preextinction training MK-801 (an NMDA antagonist) enhanced extinction (a pattern opposite to that seen in studies of

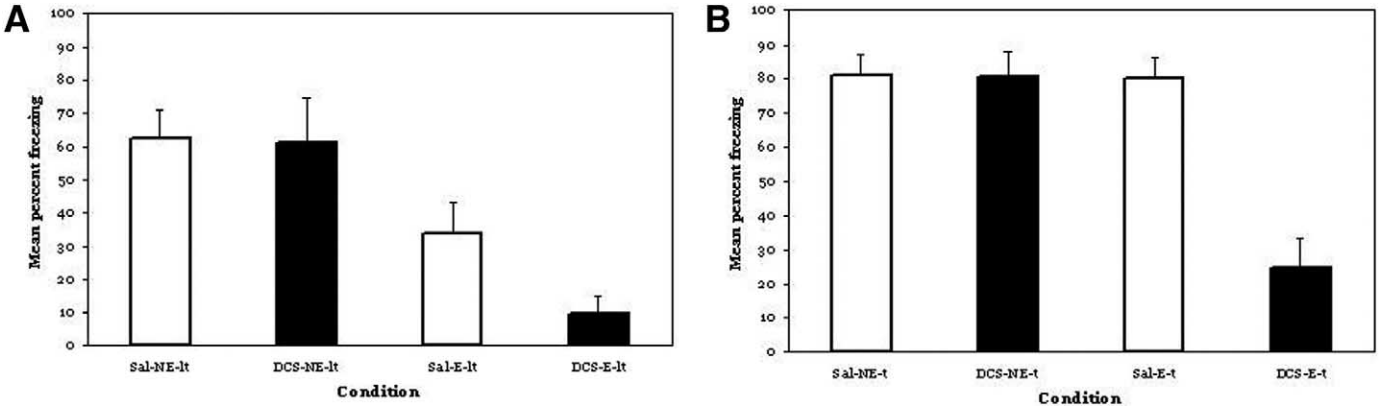

Figure 2. Effect of D-cycloserine (DCS) on conditioned freezing during test in Experiment 2. **(A)** Mean (+ SEM) percent of time rats spent freezing during four 30-sec presentations of the light CS following either extinction training to the light, or handling. **(B)** Mean (+ SEM) percent of time rats spent freezing during four 30-sec presentations of the tone CS following either extinction training to the light, or handling. Sal, saline; NE, no extinction; E, extinction; lt, light CS; t, tone CS. Reprinted with permission from Richardson et al (2004)., Ledgerwood, and Cranney (2004), Figure 2. Copyright 2004 by Cold Spring Harbor Laboratory Press. Reprinted with permission.

learned fear). These findings relate to the extinction training session only, however (i.e., when the animals were in the drug state) and could be due to state-dependent mechanisms or the effects of these agents on appetitive motivation. Further work is clearly needed in this area.

A second novel finding in this study was that although DCS facilitates extinction of learned fear, it does not affect the animal's ability to later relearn an association between the original CS and US (Experiment 1). The rationale behind Experiment 1 was that if DCS did not interfere with relearning of an association between the original stimuli, then it was unlikely to interfere with new learning using new stimuli. Further studies are needed to check this assumption explicitly, and in particular to explore the possibility of differential reactivation of the original stimulus representations. Interestingly, our results extend a previous finding (Ledgerwood et al 2004) that DCS administration does not affect the animal's ability to later learn an association between a novel context and shock.

Clinically, this second novel finding from Experiment 1 is of significant value. Although it is important that extinction-based therapies for human anxiety disorders reduce fear responding to a point whereby the patient can function in a conventional way in his or her environment, it would not be adaptive to render the patient totally incapable of acquiring new fears. Importantly, then, although the facilitation of extinction by DCS affects the original fear memory, it does not interfere with the subsequent learning of fear.

The third novel finding of this study was that DCS, administered after extinction training with one CS, led to a reduction in fear to a second CS that had been paired with the same aversive US but not extinguished (Experiment 2). In other words, rats given saline after extinction training with a light CS continued to respond to an auditory CS that had been previously paired with the same aversive US, but rats given DCS following the light extinction training failed to respond normally to the auditory CS. That is, the rats given DCS exhibited generalized extinction. This experiment needs to be replicated with a counterbalanced design, although other (non-DCS) work by Chen (2004), employing a counterbalanced discrimination procedure with the same light and tone CS as in the current study (and a shock US), showed similar levels of conditioning to the CS+, similar discrimination, and similar effects of CS+ extinction on the CS− and CS+, across the two CS modalities.

As noted earlier, several theoretical mechanisms have been proposed to explain extinction. According to what is probably the most widely accepted current account, extinction is due to a competing "context-mediated" inhibitory association (i.e., CS–no US) masking the originally acquired CS–US association (Bouton 1991). The finding that DCS-treated rats exhibit generalized extinction does not support this view. That is, the inhibitory CS–US association produced by the extinction training would be expected to be specific to that CS (e.g., compare the performance of the saline-treated rats in Experiment 2 on the extinguished and nonextinguished CSs). Therefore, our results suggest that DCS facilitates extinction of learned fear through some other process.

One possibility is that DCS facilitates extinction of conditioned fear through the process of US devaluation, a mechanism of extinction proposed by Rescorla and his colleagues (e.g., Rescorla, 1973; Rescorla and Heth, 1975). Specifically, Rescorla (1973) showed that reductions in the US representation (e.g., achieved by a procedure of US habituation) lead to reduced levels of responding to a CS that has been previously paired with that US. Unpublished data from our own laboratory have replicated this finding. Rescorla and Heth (1975) suggested that a similar deterioration of the US representation could occur following CS-only extinction training. According to these authors, when the CS is presented during extinction training, it elicits a representation of the US. However, given that no US is presented, the animal devalues the US representation. Following several CS-only presentations, the CS elicits a devalued US representation, one that is incapable of eliciting learned fear responses. The CS–US association, therefore, remains intact, but now the CS activates a US representation that is too weak to elicit fear responses. One limitation of the Rescorla and Heth explanation for our results is that if such a process were to occur during extinction training, it must somehow be specific to the CS presented during that training. That is, extinction training with one CS rarely leads to a loss in responding to a different CS previously paired with the same US (e.g., Richards and Sargent 1983; compare light- and tone-elicited performance of our saline-treated rats in Experiment 2). Administration of DCS, therefore, may not only enhance the rate at which the US representation is devalued (which leads to a faster rate of extinction), it may also reduce the CS specificity of this process. Thus, whereas CS-only extinction training appears to lead to a stimulus-gated devalued US representation, the US habituation procedure leads to a general devaluation of the US representation. DCS administration following CS-only extinction training may therefore lead to a more general devaluation of the US representation.

Although the mechanism underlying DCS facilitation of extinction may be a general devaluation of the US representation (and this certainly seems to account nicely for the results reported here), there remain several important considerations. First, it is necessary to measure more sensitively the rate of reacquisition of conditioned freezing to the light CS by DCS- and saline-treated rats. In Experiment 1, DCS-treated rats appeared to reacquire CS–US associations as well as saline-treated rats after only a single session of conditioning (i.e., 7 CS–US presentations). This finding may appear to contradict our US devaluation hypothesis, but only if one assumes that the similar low level of freezing in saline animals after two extinction training sessions are the result of mechanisms other than US devaluation, and that these other mechanisms would have no impact on subsequent relearning. Clearly, this issue requires further exploration, particularly by using a different CS (and perhaps US) from that used for original conditioning and extinction training during the reacquisition phase (see McSweeney and Swindell, 2002).

Second, it remains to be determined whether the findings reported in Experiment 2 (i.e., generalized extinction) will occur if different USs are paired with the two CSs (e.g., CS1: loud white-noise burst; CS2: shock). Indeed, perhaps the best way to test the generality of this finding and to gain additional information about the effects of DCS on extinction may be to first determine whether DCS facilitates extinction of an appetitive CS. Such research would not only determine the generality of the DCS enhancement of extinction effect, but also provide a perfect situation for testing the limitations of the generalized extinction effect. That is, in this case, rats could be given pairings of CS1 (food) and CS2 (shock). One CS could then be extinguished and the rats injected with either saline or DCS. If DCS facilitates extinction of both appetitive and aversive associations, then the rats should respond less to the extinguished CS when given DCS than when given saline. However, because these USs have different affective and sensory properties (cf. Wagner and Brandon 1989), one might not observe any generalized extinction (i.e., responding to the nonextinguished CS should be the same in these rats as in rats not given extinction training with either CS).

Third, it is possible that DCS may be affecting extinction in general and extinction generalization in particular by disrupting sensory discrimination. That is, DCS-treated animals may be overgeneralizing from the light to the tone.[1] This alternative explanation deserves testing. Fourth, it is possible that context-specific DCS effects may have mediated the apparent extinction generalization reported in Experiment 2. All phases of the experiment were conducted in the same experimental context, and although precue freezing levels were equally low during test in all groups, it is possible that fear of context (below the threshold for generating freezing) may have contributed to the freezing elicited by the compound of context plus cue.[1] In particular, the extinction generalization effect may have resulted from a more effective extinction of the context in the DCS-treated, compared with saline-treated, animals. If this were so, however, one might have expected this same mechanism to result in retarded reacquisition in Experiment 1, which it did not. Nevertheless, this possibility requires further investigation by explicit manipulation of contexts in future experiments.

Finally, we have other data that raise some problems for the US devaluation account. In a separate study (Ledgerwood et al 2004), we found that rats given DCS following CS-only extinction training failed to exhibit the US reinstatement effect. If DCS facilitates extinction by enhancing the devaluation of the US representation, then re-presenting the shock US before test should restore the US representation and therefore restore responding to the CS (e.g., Rescorla and Heth 1975). This was not the case. Although saline-treated rats (given extra extinction trials to equate levels of responding) exhibited a reliable US reinstatement effect, DCS-treated rats consistently failed to do so. This finding makes it difficult to conclude that DCS facilitates extinction by somehow enhancing the devaluation of the US representation. Therefore, although it is clear that DCS facilitates extinction of learned fear (i.e., this study; Ledgerwood et al 2003, 2004; Parnas et al, in press; Walker et al 2002), no single mechanism appears to explain adequately the effects of DCS on extinction. This may be because there several mechanisms could contribute to decreases in conditioned responding following nonreinforced exposures to the CS; that is, extinction could be due to both a devaluation of the US representation (Rescorla 1973; Rescorla and Heth 1975) and the formation of a contextually gated inhibitory association between the CS and US representations (Bouton 1991), not to mention alternative theoretical mechanisms such as an alteration of CS processing (Mackintosh 1975) or unlearning of the excitatory CS–US association (Kehoe 1988). Indeed, in a recent study on savings effects in classical conditioning of the rabbit nictitating membrane response following massive extinction, Weidemann and Kehoe (2003) reported several findings that could not be explained by any single theoretical mechanism. Therefore, Weidemann and Kehoe (2003) proposed two "hybrid" models of extinction that each accounted for the data better than any single mechanism model. Perhaps some sort of "hybrid" model for extinction of learned fear will also be better able to account for the effects of DCS.

Whatever the final determination of the mechanism by which DCS enhances extinction, it is clear from this and previous studies (Ledgerwood et al 2003, 2004; Walker et al 2002) that DCS may have significant clinical value. Evidence that the extinction of conditioned fear memory is not only facilitated by the actions of DCS, but that such action also extends to another CS previously associated with the original aversive US, has the potential to enhance the efficiency and effectiveness of current extinction-based therapies for human fear and anxiety-related disorders. In addition, it is reassuring to know that facilitation of extinction by DCS does not interfere with subsequent fear learning. The administration of DCS following an extinction-based treatment session may therefore have considerable clinical benefit (Richardson et al 2004).

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