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D-Cycloserine and the Facilitation of Extinction of Conditioned Fear: Consequences for Reinstatement

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Several recent studies have reported that D-cycloserine (DCS), a partial *N*-methyl-D-aspartate agonist, facilitates extinction of learned fear in rats. Other studies have shown that representation of the unconditioned stimulus (US) can reinstate learned fear after extinction. This study examined whether this reinstatement effect occurs in Sprague–Dawley rats given DCS at the time of extinction. Results showed that saline-treated rats exhibited the reinstatement effect but DCS-treated rats did not (Experiments 1 and 2). This lack of reinstatement in DCS-treated rats was not due to residual effects of DCS on either US or context processing (Experiment 3). Overall, these results (a) raise questions about the mechanisms underlying DCS facilitation of extinction and (b) suggest that DCS might have substantial practical benefit.

Although much is understood about the behavioral and neural mechanisms of fear acquisition, less is known about the mechanisms of fear inhibition. One way of studying the inhibition of fear in the laboratory involves the extinction procedure. In this procedure the conditioned fear response, previously acquired by pairing the conditioned stimulus (CS; e.g., light) with a fear-inducing unconditioned stimulus (US; e.g., mild shock), is reduced in both magnitude and frequency as a consequence of repeatedly presenting the CS alone. Although extinction has been the subject of study for nearly 100 years, very little is known about the mechanisms involved.

One thing that does seem to be clear, however, is that the *N*-methyl-D-aspartate (NMDA) receptor, an excitatory amino acid receptor subtype, plays a critical role in extinction of learned fear (Myers & Davis, 2002, provide an extensive review of this literature). For example, Falls, Miserendino, and Davis (1992) reported that infusion of d,1-2-amino-5-phosphonovaleric acid (AP5), an NMDA antagonist, into the basolateral nucleus of the amygdala (BLA) prior to extinction training dose-dependently blocked extinction of conditioned fear, as measured by fear-potentiated startle. Further, extinction of conditioned analgesia was blocked by systemic administration of +}-10,11-dihydro-5-methy-5H-dibenzo[ad]cycloheptene-5,10 imine (MK-801; Cox & Westbrook, 1994), and extinction of conditioned freezing was blocked by systemic administration of d(-)-3(2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP; Quirk, Rosaly, Romero, Santini, & Muller, 1999; Santini, Muller, & Quirk, 2001); both of these agents are NMDA antagonists.

Given that NMDA antagonists block extinction, one might expect that NMDA agonists would facilitate extinction. One problem here, however, is that competitive NMDA receptor agonists usually lead to excitotoxicity, and thus cell death. This difficulty is circumvented by d-4-amino-3-isoxazolidone (D-cycloserine [DCS]), a partial agonist that acts at the strychnine-insensitive glycine-recognition site of the NMDA receptor complex. This agent does not appear to lead to excitotoxicity in either rats or mice (Baran, Gramer, & Loscher, 1995; Wlaz, Baran, & Loscher, 1994). Therefore, Walker, Ressler, Lu, and Davis (2002) used this drug and found that either systemic administration or BLA infusions of DCS before extinction training enhanced the extinction of learned fear in rats, as measured by fear-potentiated startle. They also reported that DCS enhanced extinction in a dose-dependent manner, and only in rats that received extinction training (i.e., the lower levels of fear at test in the DCS-treated rats wasn't merely a consequence of being exposed to the drug). Ledgerwood, Richardson, and Cranney (2003) replicated these findings, using freezing as the measure of learned fear, and also demonstrated that DCS enhanced extinction when administered immediately after extinction training. Taken together, these results suggest that DCS enhances the extinction of conditioned fear by acting on acquisition and/or consolidation processes. The effect of DCS on consolidation processes is further supported by the finding that increasing the delay of DCS administration post-extinction training led to a linear decrease in the enhancement effect (Ledgerwood et al., 2003).

The above findings with DCS not only have implications for understanding the neural mechanisms involved in extinction, but also have potential clinical implications, particularly for individuals suffering from fear- and anxiety-related disorders such as phobias, posttraumatic stress disorder, obsessive–compulsive disorder, and panic disorder. Current behavioral treatments for these 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 Davis (see Barad, Davis, Quirk, & Phelps, 2003). In that study, it was reported that DCS

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(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 given only 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 more fully explore some of the consequences of using DCS to facilitate extinction of fear. For example, in the clinic, two common problems with current extinction-based behavioral therapies are that treatment effects are often diminished in a novel environment and after reexposure to a fearful or stressful event. These problems are modeled in the laboratory by renewal and reinstatement, respectively. That is, there are numerous reports demonstrating that an extinguished conditioned response (CR) reappears when testing occurs in a context different from that used for extinction training (i.e., renewal; Bouton & Bolles, 1979; Westbrook et al., 1995). There are also numerous reports showing that reexposure to the US alone after extinction training can restore fear of an extinguished CS (reinstatement; Bouton, 1991; Richardson, Duffield, Bailey, & Westbrook, 1999). Together, these phenomena indicate that some portion of the CR-producing association remains intact after extinction (i.e., it has merely been reduced or inhibited; see Falls, 1998). The present study is our initial attempt to explore the effects of DCS on these extinction-related phenomena.

More specifically, in the current study, we examined whether the reinstatement effect occurs in rats given DCS at the time of extinction training. In other words, given that DCS facilitates the rate at which extinction occurs (Ledgerwood et al., 2003; Walker et al., 2002), does it also have an effect on the occurrence of reinstatement? The results of this type of analysis should provide useful information for determining (a) the process by which DCS facilitates extinction, and (b) the potential clinical value of this agent in treating anxiety-related disorders.

General Method

Subjects

Adult male Sprague–Dawley rats (Gore Hill, Sydney, Australia) weighing between 300 and 500 g were used. The rats were housed in groups of 8 in plastic boxes (67 cm long × 40 cm wide × 22 cm high) in a colony room maintained on a 12-hr light–dark cycle. Food and water were continuously available. All 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. A total of 104 rats were used.

Apparatus

Rats were preexposed and conditioned in one of four standard conditioning chambers (20 cm long × 12 cm wide × 12 cm high), which were designated as Context A. 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. Unscrambled 50-Hz AC shock from a constant current generator (constructed at the University of New South Wales) could be delivered to the floor of each chamber. The chambers were contained in pairs in two sound-attenuating wooden cabinets, and each chamber was separated from its partner by a solid wooden partition within each cabinet. Each of the chambers could be viewed through a Perspex window in the front door of each cabinet. 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 0.5% acetic acid (in tap water), and the alternate two with 1.0% vanilla (in tap water). For Experiment 2 only, in the two chambers wiped with vanilla, (a) the ceiling and rear wall were dotted with round self-adhesive fasteners, 23 mm in diameter; (b) the speakers on either side of the chamber had a row of LEDs that were switched on; and (c) steel gauze was attached to the side walls. In the two chambers wiped with acetic acid, (a) the ceiling and rear wall were lined with thin black cardboard; and (b) the speakers on either side of the chamber had a row of LEDs that were covered and not switched on. All programming, timing, and shock stimulus presentations were computer controlled.

For extinction training and extinction test sessions, the context (Context B) consisted of two white-painted wooden sound-attenuating cabinets (52 cm long × 58 cm wide × 63 cm high), both of which contained a single chamber (30 cm long × 23 cm wide × 35 cm high). The front walls of these chambers were made of Perspex, the hinged lids were made of wood, and the side and rear walls were constructed of steel and painted with 2-cm wide black-and-white vertical stripes. The floors of the chambers consisted of stainless steel rods, 2 mm in diameter, spaced 13 mm apart (center to center). Below each floor was a tray containing bedding material and 1 ml of eucalyptus solution. A Perspex window in the front door of each cabinet enabled viewing of the chambers. An extractor fan was mounted on the back wall of each of the cabinets to provide a masking noise. Illumination was provided by a 15-W red light bulb located inside the door of each cabinet.

Some rats in Experiments 1 and 2 were reexposed to the US before test in Context B. Here, the US (i.e., unscrambled 50-Hz AC shock) was delivered through the floor of each chamber from a constant current generator (constructed at the University of New South Wales). A third context (Context C) was used for the US reexposure session for some rats in Experiment 2. Context C consisted of the four conditioning chambers used for Context A, but rats were exposed to the alternate Context A chamber as Context C. That is, if rats were initially conditioned in the vanilla-scented Context A, then the acetic acid-scented chamber was used as Context C and vice versa. All programming, timing, and shock stimulus presentations were computer controlled.

The CS was produced by a white light globe, and the intensity of the CS across contexts was equivalent (approximately 16–17 lx). During the experiments, the rats were observed, and their behavior was recorded with a video camera and recorder.

Drug Administration

DCS (Sigma-Aldrich, Castle Hill, New South Wales, Australia) was freshly dissolved in sterile isotonic saline (0.9% wt/vol) and injected subcutaneously in a volume of 1.0 ml/kg and a dose of 15.0 mg/kg. Control rats were injected with saline (0.9% wt/vol) in a volume of 1.0 ml/kg. The drug dose was chosen on the basis of the results of other behavioral studies (Land & Riccio, 1999; Ledgerwood et al., 2003; Pussinen et al., 1997; Walker et al., 2002) and estimates of brain concentration after systemic administration (Loscher, Wlaz, Rundfeldt, Baran, & Honack, 1994).

Behavioral Procedures

Rats were handled for 2 min a day for at least 3 consecutive days and assigned to weight-matched groups before the start of each experiment.

The general behavioral procedure is described below, and the experimental designs for each of the three experiments are shown in Tables 1 and 2.

For Experiments 1 and 2, on Day 1 (preexposure), each rat was transported to the laboratory and placed into Context A for 30 min. On Day 2 (fear conditioning), each rat was returned to Context A and trained with shock (US) signaled by a visual (light) cue (CS). Two minutes after rats were placed in the dark chamber, the CS was presented for 10 s and co-terminated with a single 0.8-mA, 0.8-s shock delivered through the grid floor of the chamber. There were five light–shock presentations, with an intertrial interval of 60 s. After the fifth light–shock pairing, each rat remained in the chamber for an additional 50 s before being returned to its home cage. On Day 3 (extinction training), rats were placed into Context B for a total of 24 min. Two minutes after the rat was placed in the dark chamber, the CS was presented for 2 min. The CS was presented six times during the 24-min session, with a 2-min intertrial interval. No shock was delivered during this session. On Day 4 (extinction training–context exposure), rats given saline after the first extinction training day (i.e., Day 3) were returned to Context B and given a 2nd day of extinction training. Rats given DCS after the 1st day of extinction training were simply returned to Context B for an equivalent period of time on Day 4 (i.e., no CS extinction trials given). On Day 5 (US reexposure), rats were placed into either Context B or C for 10 min; some of these rats received a single 0.5-mA, 0.5-s shock, which was delivered after 5 min. On Day 6 (test), the rats were exposed to Context B for 10 min, and the CS was presented for 30 s on four occasions: 6, 7, 8, and 9 min after placement in a chamber.

For Experiment 3, on Day 1 (injection), rats were transported to the laboratory and injected with either DCS or saline. On Day 2 (fear conditioning), each rat was placed into Context A and, after 2 min, given either a single 0.5-mA, 0.5-s shock or a single 0.8-mA, 0.8-s shock. After the shock, each rat remained in the chamber for an additional 50 s before being returned to its home cage. On Day 3 (test), each rat was returned to Context A for 5 min.

Scoring and Statistics

Each rat was scored for freezing during extinction training and test. Freezing was scored as the absence of all movement except that related to respiration (Fanselow, 1994). The behavior of each rat was videotaped, and freezing was rated with a time-sampling procedure in which each rat was observed once every 2 s. A percentage score was calculated. A second observer, who was unaware of the rats' group designations, rated a proportion of the sessions; the interrater reliability on the scores in all experiments was high, producing correlation coefficients greater than .96 in each experiment. Analysis of variance (ANOVA) was the primary statistical approach used to analyze extinction training and test data, with alpha set at .05.

Experiment 1: Restoration of Fear Following Representation of the US (Reinstatement)

Method

A total of 32 rats were allocated to three weight-matched groups, saline–R (reinstatement, $n = 11$); saline–NR (no reinstatement, $n = 11$); and DCS–R (reinstatement, $n = 10$). All rats received preexposure (Day 1), fear conditioning (Day 2), and extinction training (Day 3), followed immediately by DCS or saline administration (see Table 1). On Day 4, saline groups received an additional extinction training session, without saline administration. Previous work from this laboratory using a single extinction training session has shown the level of freezing of saline-treated rats at test to still be quite high, making it difficult to detect a reinstatement effect (Ledgerwood et al., 2003). To prevent this, and to provide an equivalent baseline for comparison of reinstatement between the saline and DCS groups, we therefore gave saline rats an additional extinction training session (pilot work had demonstrated that this led to equivalent levels of freezing in the two conditions). Rats given DCS after the first extinction training session were exposed to the context for only 24 min on Day 4. On Day 5, all rats were exposed to Context B for 10 min. For rats in the saline–R and DCS–R groups, a single 0.5-mA, 0.5-s shock was delivered through the grid floor 5 min after placement in the chamber. No shock was delivered to the saline–NR group. The intensity of the shock at reexposure was less than that for fear conditioning, thus avoiding high levels of freezing to the context on test. On Day 6 (test), each rat was placed in Context B for 10 min, and the CS was presented for 30 s on four occasions: 6, 7, 8, and 9 min after placement in the chamber (following the procedure of Westbrook, Iordanova, McNally, Richardson, & Harris, 2002).

Results

During the first 2 min of extinction training, before the first light CS presentation (precue), there were no group differences in levels of freezing (means ranged from 7.31% to 12.59%). Freezing during the first presentation of the light CS was considerable

Table 1
Summary of Design for Experiments 1 and 2

Group	Exposure (Day 1)	Conditioning (Day 2)	Extinction (Day 3)	Extinction (Day 4)	Reinstatement (Day 5)	Test (Day 6)
			Experiment 1			
Sal-NR	A	A: CS-US	B: CS-Sal	B: CS	B: no US	B: CS
Sal-R	A	A: CS-US	B: CS-Sal	B: CS	B: US	B: CS
DCS-R	A	A: CS-US	B: CS-DCS	B	B: US	B: CS
			Experiment 2			
Sal-S	A	A: CS-US	B: CS-Sal	B: CS	B: US	B: CS
Sal-D	A	A: CS-US	B: CS-Sal	B: CS	C: US	B: CS
DCS-S	A	A: CS-US	B: CS-DCS	B	B: US	B: CS
DCS-D	A	A: CS-US	B: CS-DCS	B	C: US	B: CS

Note. A, B, and C refer to three distinct contexts. Sal = Saline; NR = no shock unconditioned stimulus (US) reinstatement; CS = conditioned stimulus; DCS = D-cycloserine; R = US reinstatement; S = test context same as US reinstatement context; D = test context different from US reinstatement context; no US = absence of shock. A light was used as the CS.

Table 2
Summary of Design for Experiment 3

Group	Drug (Day 1)	Conditioning (Day 2)	Test (Day 3)
Sal-0.5	Sal	A: US	A
Sal-0.8	Sal	A: US	A
DCS-0.5	DCS	A: US	A
DCS-0.8	DCS	A: US	A

Note. A refers to context. Sal = Saline; DCS = D-cycloserine; 0.5 = 0.5-mA, 0.5-s footshock unconditioned stimulus (US); 0.8 = 0.8-mA, 0.8-s footshock.

(overall $M = 52.89\%$, $SD = 30.32$), but then fell gradually across successive cue presentations (overall *M* level of freezing on Trial 6: $M = 0.48\%$, $SD = 1.62$), demonstrating that extinction of fear to the light CS occurred in the short term, $F(5, 145) = 35.35$.

At test, the level of freezing during the 30-s period prior to the initial CS presentation varied somewhat between groups, with the reinstated groups exhibiting more freezing than the non-reinstated group (saline–R: $M = 19.95\%$, $SD = 31.28$; saline–NR: $M = 0.91\%$, $SD = 3.02$; DCS–R: $M = 24.75\%$, $SD = 41.34$). However, ANOVA failed to detect any significant group differences, $F(2, 31) = 1.95$, $p = .16$. The mean levels of freezing averaged across the four CS presentations during test are shown in Figure 1. An ANOVA revealed a significant group difference, $F(2, 31) = 4.38$, and subsequent pairwise comparisons, with Tukey's honestly significant difference test, revealed that the saline–R rats froze significantly more than both the saline–NR and DCS–R rats, which did not differ. The performance of the saline rats replicates well-documented findings that reexposure to the US will reinstate an extinguished CR (Bouton & Bolles, 1979; Rescorla & Heth, 1975). The novel result in this experiment was that reinstatement was not evident in the rats administered DCS after extinction training.

The results of this experiment suggest that rats given DCS at the time of extinction fail to exhibit the reinstatement effect. That is, although the DCS-treated rats were reexposed to the US before test, their performance was significantly poorer than that of the saline-treated rats reexposed to the US and identical to that of saline-treated rats not reexposed to the US before test. However, it must be acknowledged that this interpretation of the results is based on accepting the assumption that giving saline-treated rats two extinction sessions produces a level of fear equivalent to that seen in DCS-treated rats after a single extinction session. In other words, it is possible that DCS-treated rats not given the US before test would have exhibited levels of freezing less than that exhibited by the saline–NR group in this experiment (i.e., the DCS–R group in this experiment would actually be exhibiting a reinstatement effect if they were compared with a DCS–NR group). This possibility is not supported by comparisons of the data reported in the present experiment with that reported in other experiments from this laboratory. Ledgerwood et al. (2003), using the same procedures as those used in the present study, reported the results of five experiments examining the effects of DCS on extinction of learned fear in rats. In each of the three experiments that involved systemic administration of 15 mg/kg DCS, there was a DCS-treated group that received a single extinction training session, and no reinstatement treatment before test. The mean levels of freezing exhibited at test by these three groups were 18.23%, 7.01%, and 14.85% (in Experiments 1, 2, and 4, respectively). These levels of freezing are very similar to those exhibited by both the DCS–R rats ($M = 15.78\%$) and the saline–NR animals ($M = 17.61\%$) in the present experiment. In other words, the performance of the DCS–R rats in the present experiment is very similar to that exhibited by DCS-

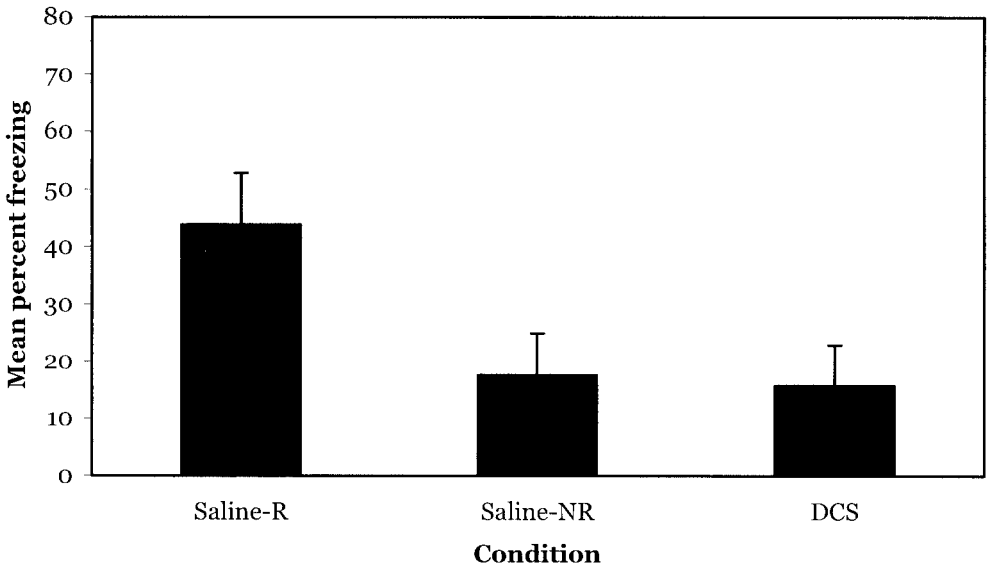

Figure 1. Effect of D-cycloserine (DCS) on conditioned freezing during the postextinction test after unconditioned stimulus (US) reinstatement. Mean (+ *SEM*) percentage of time rats spent freezing during four 30-s presentations of the light conditioned stimulus in Experiment 1. NR = no US reinstatement; R = US reinstatement.

treated rats not reexposed to the US before test. Further, the performance of saline-treated rats given two extinction sessions is also very similar to that seen in DCS-treated rats given a single extinction session (for further comparison purposes, it should be noted that saline-treated rats given a single extinction training session exhibit substantially higher mean levels of freezing at test: 44.56%, 41.63%, and 75.09%, in Experiments 1, 2, and 4, respectively, from Ledgerwood et al.).

Experiment 2: Context Modulation of Reinstatement

In Experiment 1, conditioned responding to an extinguished CS was reinstated when rats were reexposed to the US after extinction training. This was the case, however, only for rats administered saline immediately after extinction training. Rats administered DCS immediately after extinction training failed to exhibit reinstatement. This finding with DCS-treated rats is both novel and surprising. It is important then, to determine the reliability of these results. As noted earlier, extinction-based treatment effects for human fear disorders are often diminished after exposure to an aversive, or stressful, stimulus. Therefore, if a treatment can be found that not only enhances extinction of learned fear but also prevents the reinstatement of that fear (i.e., relapse), then it would be of considerable clinical value.

Experiment 2, therefore, set out to replicate, and extend, the results obtained in Experiment 1. Previous research on reinstatement has shown that this effect is context specific. That is, reexposure to the US prior to test restores responding in that context, but not others (e.g., Bouton & Bolles, 1979; Westbrook et al., 2002). Therefore, in this experiment we manipulated the context in which the US was presented before test. Specifically, some rats were reexposed to the US in the context in which they would be tested, whereas others were reexposed to the US in a context different from that where they would be tested. On the basis of work by Bouton and Bolles (1979) and Westbrook et al. (2002), we predicted that saline-treated rats would exhibit reinstatement of fear only if the US was presented in the test context (i.e., reinstatement would be context specific). On the basis of the findings of Experiment 1, we also predicted that DCS-treated rats would not exhibit any reinstatement effect, regardless of where the US had been presented.

More specifically, rats in Experiment 2 were conditioned in one context (A), extinguished in a second context (B), reexposed to the US in either Context B or a third context (C), and finally, tested in Context B. In other words, this experiment had a 2 (postextinction drug: DCS or saline) $\times$ 2 (context of US reexposure: same as testing or different from testing) design. It should be noted that inclusion of the DCS–different condition in the present study can be seen as providing a control group that was not explicitly included in Experiment 1. That is, Experiment 1 did not contain a DCS–NR control; we relied on comparisons from our previous work for this. In the present study, however, the DCS–different condition can be seen as an even more stringent control than that offered by a DCS–NR group. Rats in the two *different* groups (i.e., saline- and DCS-treated) will be exposed to the US before test, but in a manner that would not normally be expected to lead to restoration of fear. If the DCS-treated rats actually exhibit reinstatement, when compared with an appropriate control, then this experiment will reveal this (i.e., rats in the DCS–same condition should perform at higher levels than rats in the DCS–different condition).

Method

Forty rats were allocated to four weight-matched groups: saline–same (n = 11), saline–different (n = 11), DCS–same (n = 9), and DCS–different (n = 9). All rats received preexposure (Day 1), fear conditioning (Day 2), and extinction training (Day 3) with subsequent DCS–saline administration (see Table 1). On Day 4, saline groups received an additional extinction training session, whereas DCS rats were placed in Context B for an equivalent period but did not receive exposures to the light CS. On Days 3 and 4, at least 2 hr before each extinction training session, all rats received a 24-min exposure to Context C, without CS presentations. On Day 5, rats were exposed to either Context B (groups: saline–same and DCS–same) or Context C (groups: saline–different and DCS–different) for 10 min. All rats received a single shock (0.5 mA, 0.5 s) 5 min after placement in the context. On Day 6, rats received the same test as that described in Experiment 1.

Results

During the first 2 min of extinction training, before the first light CS presentation (precue), there were no group differences in levels of freezing (means ranged from 3.50% to 4.20%). Freezing during the first presentation of the light CS was considerable (overall M = 48.66%, SD = 31.37), but then fell gradually across successive cue presentations (overall level of freezing on Trial 6: M = 0.58%, SD = 1.64), demonstrating that extinction of fear to the light CS occurred in the short term, $F(5, 180) = 45.96$.

At test, the level of freezing during the 30-s period before the initial CS presentation varied somewhat between groups, with the groups reinstated in the same context as that used for extinction training and test exhibiting more freezing than the groups reinstated in a different context (saline–same: M = 9.47%, SD = 10.52; DCS–same: M = 3.33%, SD = 5.00; saline–different: M = 0.00%, SD = 0.00; DCS–different: M = 2.22%, SD = 4.41). However, ANOVA failed to detect any significant group differences, $F(3, 39) = 2.63$, $p = .07$. The mean levels of freezing averaged across the four CS presentations during test are shown in Figure 2. An ANOVA revealed a significant group difference, $F(3, 39) = 6.81$, and subsequent pairwise comparisons, with Tukey's honestly significant difference test, revealed that the saline–same rats displayed significantly higher levels of freezing than the saline–different, DCS–different, and DCS–same rats; the performance of the latter three groups did not differ.

In summary, reexposure to the US in the extinction training context restored freezing to the CS when saline-treated rats were tested in that context (group: saline–same). This effect did not occur when rats were reexposed to the US in a context different from that of extinction training and test (group: saline–different). These results are consistent with the work of Bouton and Bolles (1979) and Westbrook et al. (2002). In contrast, DCS-treated rats failed to exhibit reinstatement, regardless of where US reexposure occurred. The findings of Experiment 1 were thus replicated, and it appears that the lack of reinstatement in DCS-injected rats is a reliable effect.

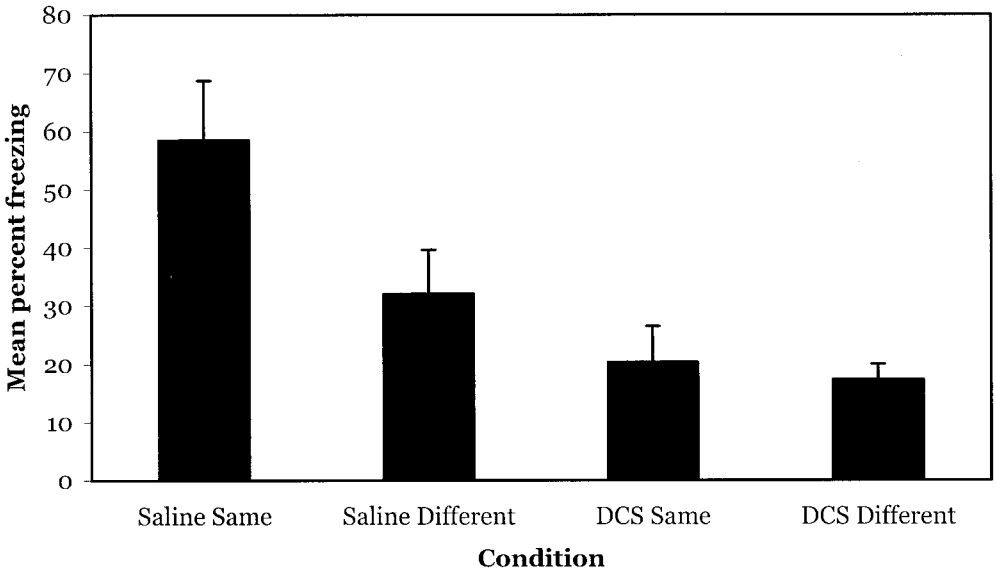

Figure 2. Effect of D-cycloserine (DCS) on conditioned freezing during the postextinction test after unconditioned stimulus reinstatement in either the same or a different context from that of extinction training. Mean (+ *SEM*) percentage of time rats spent freezing during four 30-s presentations of the light CS in Experiment 2. Same = same context as reinstatement; Different = different context to reinstatement.

Experiment 3: Effect of DCS on Subsequent US and Context Processing

Experiments 1 and 2 show that rats injected with DCS after extinction training fail to exhibit the reinstatement effect. One way to possibly understand this finding involves considering the learning processes involved in reinstatement. Current views of extinction maintain that during extinction training, the expression of the original excitatory CS–US association is inhibited, and this inhibition is specifically related to the context in which extinction training occurs (Bouton, 1993; Rescorla, 1979). There are two primary candidate mechanisms for this inhibition. First, Rescorla and colleagues (e.g., Rescorla, 1979; Rescorla & Cunningham, 1977) located the inhibition in the context–US association, proposing that the extinction-training context increases the threshold at which activation of the US representation can occur. Second, Bouton (1993) located the inhibition in specific features of the extinction-training context that activate a CS–no-US memory. According to Bouton, information acquired about a CS across conditioning and extinction training is represented as separate CS–US and CS–no-US memories. Subsequent responding to the CS is determined by which of these memories is activated; each memory has different conditions of activation. Activation of the CS–US memory is independent of the context, whereas activation of the CS–no-US memory is explicitly connected to the extinction-training context. From either of these perspectives, reexposure to the US before test would be expected to produce context-specific reinstatement. Further, and more important for present purposes, from both of these perspectives, any long-term effects of DCS on the processing of the US or the context might lead to deficits in the reinstatement effect. Therefore, in Experiment 3 we examined whether DCS injections had any long-term effects on the processing of the US or the context by injecting rats with either DCS or saline 24 hr before contextual conditioning with a high- or low-intensity shock (see Table 2).

Method

Thirty-two rats were allocated to four weight-matched groups: saline–0.5; saline–0.8; DCS–0.5; and DCS–0.8 (*n*s = 8 per group). On Day 1 (injection), rats were transported to the laboratory and injected with either DCS or saline. On Day 2 (fear conditioning), rats were placed into Context A and, after 2 min, given either a single 0.5-mA, 0.5-s shock (groups: saline–0.5, DCS–0.5) or a single 0.8-mA, 0.8-s shock (groups: saline–0.8, DCS–0.8). Shock intensities were based on those used for conditioning and US reinstatement in the two previous experiments. After the shock, each rat remained in the chamber for an additional 50 s before being returned to its home cage. On Day 3 (test), each rat was returned to Context A for 5 min, and its level of freezing was recorded.

Results

Figure 3 shows that the levels of contextual freezing for the DCS and saline groups were equivalent (drug main effect, $F < 1$). There was, however, a significant effect of shock intensity, with levels of freezing in the groups that received the 0.8-mA, 0.8-s shock being higher than that for the groups that received the 0.5-mA, 0.5-s shock, $F(1, 28) = 4.81$. There was no significant interaction of drug and shock intensity ($F < 1$). Rats injected with DCS can, therefore, process shock and learn about contexts similarly to saline rats. In other words, the failure to observe reinstatement in Experiments 1 and 2 was not due to DCS having long-term deleterious effects on the processing of the shock US or the context.

General Discussion

The current study demonstrates that rats given the partial NMDA agonist DCS immediately after an extinction training

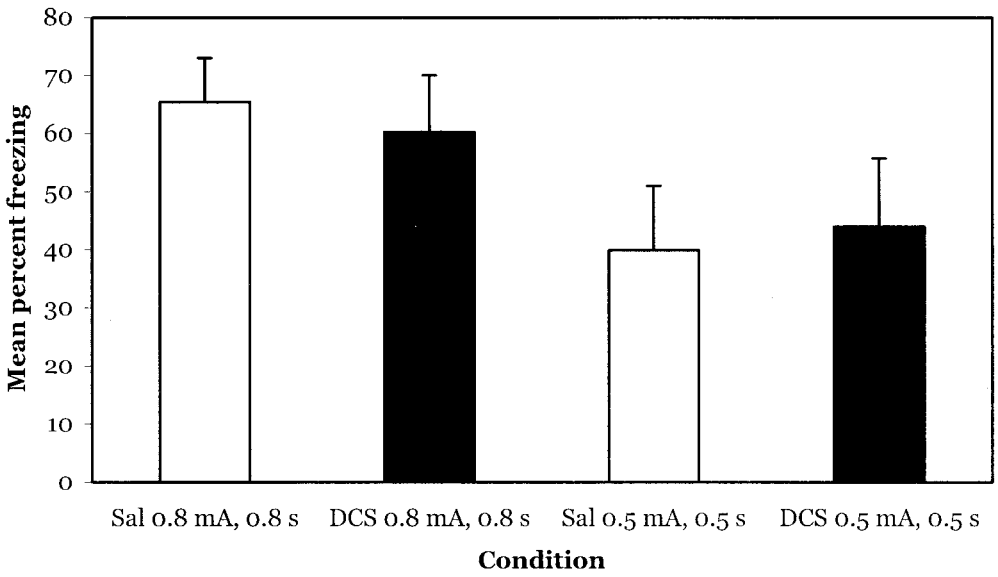

Figure 3. Effect of D-cycloserine (DCS) on conditioned contextual freezing after a single high- (0.8-mA, 0.8-s) or low- (0.5-mA, 0.5-s) intensity footshock. Mean (+ *SEM*) percentage of time rats spent freezing during one 5-min exposure to the conditioning context (i.e., Context A) in Experiment 3. Sal = saline.

session fail to exhibit reinstatement of conditioned fear after reexposure to the US (Experiments 1 and 2). In contrast, rats administered saline immediately after extinction training exhibit reinstatement after US reexposure in the same context as that used for extinction training and test (Experiments 1 and 2). Saline-treated rats do not exhibit reinstatement when US reexposure occurs in a context different from that of extinction training and test (Experiment 2). Further, the failure of DCS-treated rats to exhibit reinstatement cannot be attributed to long-term deficits in contextual or US processing after drug injection (Experiment 3).

These findings demonstrate that the use of DCS in conjunction with extinction-based behavioral theories is potentially of significant clinical value. Systematic desensitization, flooding, and implosion are all examples of extinction-based therapeutic techniques used to treat human fear- and anxiety-related disorders. As previously mentioned, however, one problem with these techniques is that reexposure to a fearful, or stressful, event can result in reinstatement (see Bouton & Nelson, 1998). DCS, shown here to prevent reinstatement in rats when administered immediately after a single extinction training session, thus has the potential to enhance the effectiveness and efficiency of current treatments for human fear disorders not only by facilitating extinction (Ledgerwood et al., 2003; Walker et al., 2002), but also by preventing relapse after a stressful experience.

The results of the present study also raise some interesting theoretical questions concerning how DCS operates in order to facilitate extinction. Current psychological theories of extinction can be divided into two general categories. The first category views extinction as a process of "unlearning," suggesting that during extinction training, the excitatory CS–US association is weakened and eventually erased, such that the CS no longer elicits the original CR (e.g., Rescorla & Wagner, 1972; Wagner & Rescorla, 1972). The second category proposes that extinction involves the formation of new associations that compete with, or mask, the expression of the original CS–US association (Bouton, 1991; Konorski, 1948; Mackintosh, 1975). A particularly noteworthy example of the new-learning view of extinction comes from the work of Bouton and colleagues (e.g., Bouton, 1991; Bouton & Bolles, 1979; Bouton & Ricker, 1994; Brooks & Bouton, 1994). From the perspective of this theoretical approach, during extinction training, the animal encodes a second memory of the CS (i.e., CS–no US). This representation of the CS then competes with the original memory of the CS (i.e., CS–US), and the animal's level of responding is determined by which of the two representations is retrieved. An important distinction, according to Bouton's view, is that the CS–no-US representation is highly context bound, whereas the CS–US representation is much less affected by context. In other words, retrieval of the CS–no-US representation is dependent on the animal being tested in the extinction-training context. Given the role of NMDA receptors in new learning (Collingridge & Bliss, 1987; Flood, Baker, & Davis, 1990; Maren, Aharanov, Stote, & Fanselow, 1996), a reasonable hypothesis is that DCS facilitates this specific form of new learning, thus leading to enhanced extinction (see Ledgerwood et al., 2003; Walker et al., 2002). From this perspective, any manipulation that interferes with the extinction training context's "retrieval" of the inhibitory association should result in a restoration of fear responding. One such manipulation is reinstatement. Therefore, from this perspective, rats administered DCS after extinction training should exhibit reinstatement when reexposure to the US occurs in the extinction training context, thus disrupting the occasion-setting properties of that context. The results of this study, however, do not support this hypothesis. Specifically, Experiments 1 and 2 provided no evidence for reinstatement in the DCS groups after US reexposure. One possible explanation for this result could be that DCS-injected rats have developed an extremely strong inhibitory CS–US association. If this were the case, then demonstration of reinstatement might not be as straightforward in these rats as it is in saline-

treated rats. That is, it may be necessary to provide stronger, or more, US exposures in order to observe reinstatement in the DCS-treated rats. Clearly, further research is required to examine this possibility, but this sort of finding would not dramatically alter the potential clinical significance of the present findings.

Alternatively, perhaps the failure to observe reinstatement in DCS-treated rats can be better understood from the perspective of unlearning. That is, perhaps DCS facilitates extinction by actually enhancing the unlearning of the CS–US association. As noted above, certain theoretical models of Pavlovian conditioning explicitly describe extinction as an unlearning process (e.g., Rescorla & Wagner, 1972). However, this explanation of extinction has fallen somewhat out of favor given the abundant evidence that the CS–US association remains intact after extinction. Our current findings with saline-injected rats (Experiments 1 and 2), for example, demonstrate that US reexposure after extinction training restores conditioned responding (i.e., reinstatement; see also Rescorla & Heth, 1975; Westbrook et al., 2002). Further, testing animals in a context different from that used for extinction training also often leads to a restoration of responding, that is, renewal (Bouton & Bolles, 1979; Bouton & King, 1983; Bouton & Ricker, 1994). Both of these findings show that extinction does not simply involve the unlearning of the CS–US association. However, it should be noted that responding rarely returns to preextinction levels after reinstatement or renewal. In other words, it is possible that some unlearning has occurred. Perhaps DCS administration leads to greater unlearning as a result of extinction training? This explanation is consistent with studies showing that massive amounts of extinction training eliminate both renewal and reinstatement (Denniston, Chang, & Miller, 2003; Rauhut, Thomas, & Ayres, 2001). In other words, unlearning of the CS–US association may occur during extinction, but only in those cases where massive amounts of extinction training are given, or in cases where DCS is given. If this account of how DCS facilitates extinction is correct, however, then it must also be noted that this unlearning does not appear to be restricted to the CS being presented, but also occurs for other CSs that had been previously paired with a common US associate (Ledgerwood et al., 2004). Unlearning models in their raw form (see Kehoe, 1988) fail to account for generalization between CS-specific links. That is, from the perspective of these unlearning models of extinction, the extinction of one CS-specific link would not result in the extinction of another, separate CS-specific link. Therefore, it may be necessary to further modify these unlearning models to account for this apparent effect of DCS on extinction of learned fear.

Whatever the final determination of the mechanism by which DCS facilitates extinction of learned fear, the present findings have potential clinical significance. DCS, shown here to prevent reinstatement in rats when administered immediately after a single extinction training session, has the potential not only to enhance the efficacy of current treatments for human fear- and anxiety-related disorders, but also to sustain treatment effects after reexposure to the originally feared (or stressful) event, thus preventing relapse.

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