

D-Cycloserine Facilitates Extinction the First Time but not the Second Time: An Examination of the Role of NMDA Across the Course of Repeated Extinction Sessions

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Extinction of learned fear is facilitated by the partial NMDA agonist D-cycloserine (DCS). However, some studies suggest that the involvement of NMDA in learning differs depending on whether learning is for the first or second time. The current study aimed to extend these findings by examining the role of NMDA in extinction for the first and the second time. Specifically, the present series of experiments used Pavlovian fear conditioning and extinction paradigms to compare the effect of DCS on extinction of fear to a light CS the first and second time around. As found previously, DCS facilitated extinction of learned fear (Experiment 1). A novel finding, however, was that DCS did not facilitate the re-extinction of fear to this same CS following retraining (Experiments 2A and 2B). Finally, it was demonstrated that the transition from NMDA-dependent to NMDA-independent extinction was stimulus specific (Experiment 3). That is, rats were first trained to fear a CS (light); this fear was then extinguished. Following this, rats were then retrained to fear the same CS (light) or a new CS (white noise). When given a second extinction session, DCS was found to facilitate extinction of the new CS but not the original CS. The results of this series of experiments suggest that the role of NMDA in extinction depends on whether extinction is new learning (first extinction) or retrieval of a previous extinction memory (re-extinction).

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INTRODUCTION

The World Health Organization predicts that by the year 2020 depressive and anxiety disorders combined will be the second leading cause of burden among all diseases. Pavlovian fear conditioning provides an animal model of anxiety disorders. Typically, fear conditioning involves pairing a neutral stimulus such as a light 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 (eg, freezing and fear-potentiated startle). However, the fear elicited by the CS can be reduced by repeatedly presenting the CS in the absence of the US (ie, extinction).

Over the past few decades, extinction has been increasingly investigated at both the theoretical and neural level. This increased attention has been driven, at least in part, by the potential clinical importance of extinction as an animal model for exposure-based therapies for treating anxiety

disorders. Indeed, advancements made in preclinical studies of extinction are proving successful in recent clinical trials. Specifically, the partial *N*-methyl-D-aspartate (NMDA) agonist D-cycloserine (DCS), which has been shown to facilitate extinction learning in rats (Walker *et al*, 2002; Ledgerwood *et al*, 2003), has also been shown to facilitate exposure-based therapy in humans diagnosed with a range of anxiety disorders including acrophobia (Ressler *et al*, 2004), social phobia (Hofmann *et al*, 2006; Guastella *et al*, 2008), and obsessive-compulsive disorder (Kushner *et al*, 2007). Despite this progress in translational research, the phenomenon of extinction is not fully understood. There is no doubt that continued preclinical investigations of extinction have important theoretical and clinical implications (Davis *et al*, 2006; Hofmann, 2007).

In regards to theories of extinction, one early model suggested that extinction is due to 'unlearning' or 'erasure' of the CS-US association (Rescorla and Wagner, 1972), while another suggested that extinction may be due to devaluation of the US representation (Rescorla, 1973). However, a much more widely accepted view of extinction is that it is a type of new learning. Specifically, according to Bouton (1991), extinction involves the formation of a context-specific, inhibitory 'CS-no US' association, which competes with the original CS-US association. There is evidence that this new learning involves the same molecular

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and cellular mechanisms as fear conditioning. Specifically, it is well established that the formation of long-term memory requires activation of a complex molecular signaling cascade involving NMDA receptor activation as well as the phosphorylation of protein kinases such as mitogen-activated protein kinase (MAPK), protein synthesis, and gene transcription (Kandel, 2001). This is supported by studies showing that administration of NMDA (Miserendino *et al*, 1990; Goosens and Maren, 2004) and MAPK (Atkins *et al*, 1998; Schafe *et al*, 2000) antagonists impair Pavlovian fear conditioning when administered systemically or directly into the brain. These same manipulations have been found to disrupt the retention of extinction learning (Falls *et al*, 1992; Lu *et al*, 2001).

Collectively, these studies suggest that fear conditioning and extinction are types of new learning that require the action of complex molecular signaling cascades. Further, it is clear that both fear conditioning and extinction are NMDA-dependent processes. However, a number of recent studies suggest that NMDA receptors are not always required for the acquisition of learned fear. Specifically, these studies suggest that learning for the second time (relearning) may rely on different neurobiological processes compared with learning for the first time. For example, Sanders and Fanselow (2003) examined the effect of APV (NMDA antagonist) infusion into the dorsal hippocampus prior to contextual fear conditioning. As expected, infusion of APV impaired contextual fear conditioning. However, an unexpected finding was that if rats received vehicle infusion prior to conditioning in context A, and then received APV infusion prior to conditioning in context B, then fear conditioning to context B was not impaired. In other words, contextual fear conditioning in context B was rendered NMDA-independent by the prior conditioning experience in context A.

Research using one-trial inhibitory avoidance also suggests that learning for the second time requires different neural processes than initial learning. When using an inhibitory avoidance paradigm, the amount of learning is increased by giving rats a second trial. However, the extra learning produced by this second learning trial does not require activation of NMDA receptors or protein synthesis in brain regions normally involved in one-trial inhibitory avoidance (Roesler *et al*, 1998; Cammarota *et al*, 2004). Moreover, infusion of APV into the hippocampus after training revealed that hippocampal NMDA receptors were not required for learning if the subject had previously learned about the task by being pre-exposed to the training context (Roesler *et al*, 1998). Therefore, the learning occurring on the second trial of inhibitory avoidance is another case where the neural mechanisms required for learning are different from those required for learning the first time.

Therefore, it seems that learning the first time may be fundamentally different from learning the second time. The current series of experiments aimed to continue this line of investigation in relation to extinction learning with the rationale that just as there are differences between learning and relearning, there may be differences between extinction and re-extinction. Specifically, the current study examined the effect of the partial NMDA agonist DCS on extinction and re-extinction. As previously mentioned, DCS has been

found to facilitate extinction in rats (eg, Walker *et al*, 2002; Ledgerwood *et al*, 2003). On the basis of studies showing that learning the second time around may be NMDA-independent, the current study examined whether re-extinction would be facilitated by DCS administration. Understanding how extinction operates the second time around is both clinically and theoretically important as the same manipulations that facilitate extinction may not facilitate re-extinction.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (~90 days old) were used (School of Psychology, University of New South Wales, Sydney, NSW, Australia). Rats were housed in groups of eight and maintained on a 12-h light/dark cycle with food and water continuously available. All procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales.

Apparatus

Experiments 1, 2A, and 2B were conducted in a set of two identical startle cages (see Weber and Richardson, 2004 for details). The startle stimulus was a 100-ms, 100-db (measured with a Bruel and Kjaer precision sound level meter, Type 2235) white noise with 1-ms rise-fall time and was presented from two piezoelectric speakers mounted on either side of the startle cage. The CS was a 10-s white LED light located on the rear wall of the wood cabinet and the US was a 0.6-mA, 1-s footshock delivered through the stainless steel grid floor. Computer software custom developed at the University of New South Wales controlled all stimulus presentations and recorded all startle data. After each session the chambers were wiped clean with tap water.

Experiment 3 occurred in a set of two identical rectangular chambers (30 cm long × 30 cm wide × 28 cm high) wholly constructed of transparent Plexiglas, with the exception of a grid floor of stainless steel bars (spaced 1.3 cm apart). Two high-frequency speakers were mounted on the ceiling of each chamber. Each chamber was housed within a separate sound attenuating wood cabinet. The two CSs (both 10 s in duration) used in this experiment were a white LED light located on the rear wall of the wood cabinet (CS1) and white noise (CS2); noise level in the chambers was increased by 8 dB when CS2 was presented. The US was a 0.6-mA, 1-s footshock. Computer software custom developed at the University of New South Wales controlled all stimulus presentations. After each session chambers were wiped clean with tap water.

Pharmacological Treatment

D-Cycloserine (Sigma-Aldrich, Australia) was freshly dissolved in sterile saline (0.9%, wt/vol). DCS was injected subcutaneously (in the nape of the neck) at a volume of 1.0 ml/kg and a dose of 15 mg/kg; control rats were injected with saline at a volume of 1.0 ml/kg. This dose of DCS was chosen based on previous results (eg, Walker *et al*, 2002; Ledgerwood *et al*, 2003).

General Behavioral Procedures

Experiment 1. Experiment 1 used a 2×2 factorial design, in which the first factor was drug (DCS or saline) and the second factor was number of extinction trials (15 or 30). Behavioral procedures consisted of four phases, each conducted on separate days. During each phase of the experiment there was a 2-min adaptation period.

On day 1 rats received 10 CS-US pairings (mean intertrial interval 2 min). The following day, rats were tested for learned fear. Specifically, rats were given 30 habituation trials in which the startle-eliciting noise was presented. Following this, rats received 12 baseline (BL) trials where the noise was presented alone and 6 CS trials where the noise was presented in the presence of the CS; these trials were randomly intermixed. A comparison was made between average startle responses in the presence of the light (CS) and in the absence of the light (BL). This difference was calculated as mean percentage change in startle amplitude from BL using the formula $CS - BL / BL * 100$ (see Walker and Davis, 2002). Subjects were then allocated to groups so that each group was matched on levels of fear responding. On day 3, extinction training occurred. Immediately before extinction training (15 or 30 CS-alone presentations; 30 s ISI was used during extinction sessions in all experiments) rats were injected subcutaneously with saline or DCS. On day 4, subjects were tested as on day 2.

Experiment 2A. Experiment 2A used a 2×2 factorial design, in which the first factor was drug (DCS or saline) and the second factor was retraining (retrained or not retrained). Note that in experiment 2A (and subsequent experiments) rats were given 2 days of extinction training to ensure rats had low levels of fear before retraining occurred. Behavioral procedures consisted of nine phases, each conducted on separate days (see Table 1). On day 1, fear conditioning was conducted as in Experiment 1. The following day, rats were tested for learned fear as in Experiment 1 and allocated to groups so that each group was matched for initial levels of fear. On days 3 and 4, rats received extinction training (15 CS-alone presentations each day). On day 5, subjects were tested for fear of the CS (as on day 2). On day 6, animals were retrained or not: rats in the retrained group received fear conditioning (as on day 1), whereas rats in the non-retrained group were simply placed in the conditioning chambers for the same period of time. This non-retrained control group was included to ensure that any subsequent increase in responding to the CS was a

result of CS-US retraining rather than spontaneous recovery. On day 7, rats were tested for fear of the CS (as on day 2). The next day, re-extinction training occurred. Immediately before re-extinction training (15 CS-alone presentations), rats were injected subcutaneously with saline or DCS. Then on day 9, rats were tested for learned fear (as on day 2).

Experiment 2B. The procedures for Experiment 2B were the same as in Experiment 2A (see Table 1) except that rats received 30 non-reinforced extinction trials in all extinction sessions and 5 light-shock pairings at retraining instead of 10. Further, because non-retrained control rats in Experiment 2A exhibited low levels of fear at test, this control group was not included in Experiment 2B.

Experiment 3. Experiment 3 employed a 2×2 factorial design, in which the first factor was drug (DCS or saline) and the second factor was re-extinction CS (same or different CS as initial extinction). Rats were randomly allocated to one of four groups and behavioral procedures consisted of seven phases, each conducted on separate days (Table 2). Freezing, which is indicated by the absence of all movement except that required for respiration (Blanchard and Blanchard, 1969), was used as the measure of fear in this experiment. Animals were judged as freezing or not every 3 s and a percentage score was calculated to determine the proportion of freezing during the total observation period in each testing session.

On day 1, fear conditioning (five light-shock pairings) occurred. On days 2 and 3, extinction training (30 CS-alone presentations each day) occurred. On day 4, rats were tested for fear of the light CS. This test consisted of a 2-min adaptation period followed by a 3-min period of nine non-reinforced 10 s light presentations with a 10-s intertrial interval. There were no significant group differences in levels of fear during test on day 4 (largest $F(1, 27) = 0.87$, $p > 0.05$). On day 5, rats were retrained to either CS1 (five light-shock pairings) or CS2 (five white noise-shock pairings). On day 6, either CS1 or CS2 were re-extinguished (extinction consisted of 30 CS-alone presentations in both cases). Before extinction training on day 6, rats were injected subcutaneously with saline or DCS. On day 7, rats

Table 1 Design for Experiments 2A and 2B

Group	D1	D2	D3	D4	D5	D6	D7	D8	D9
Retrained SAL	CS+	FPS	CS-	CS-	FPS	CS+	FPS	SAL/CS-	FPS
Retrained DCS	CS+	FPS	CS-	CS-	FPS	CS+	FPS	DCS/CS-	FPS
Non-retrained SAL	CS+	FPS	CS-	CS-	FPS		FPS	SAL/CS-	FPS
Non-retrained DCS	CS+	FPS	CS-	CS-	FPS		FPS	DCS/CS-	FPS

CS+ denotes fear conditioning (CS-US pairings); CS- denotes extinction training (CS-alone presentations); FPS denotes testing for fear; and SAL or DCS refers to drug administered (saline or D-cycloserine).

Table 2 Design for Experiment 3

Group	D1	D2	D3	D4	D5	D6	D7
Same CS-SAL	CS1+	CS1-	CS1-	Test CS1	CS1+	SAL/CS1-	Test CS1
Same CS-DCS	CS1+	CS1-	CS1-	Test CS1	CS1+	DCS/CS1-	Test CS1
Different CS-SAL	CS1+	CS1-	CS1-	Test CS1	CS2+	SAL/CS2-	Test CS2
Different CS-DCS	CS1+	CS1-	CS1-	Test CS1	CS2+	DCS/CS2-	Test CS2

CS+ denotes fear conditioning; CS- denotes extinction training; 'Test' denotes testing for fear; and SAL or DCS refers to drug administered (saline or D-cycloserine).

were given a test (identical to day 4) to assess freezing to the same CS that the rat had been trained with on day 5.

Note that retraining and re-extinction are used as general terms to refer to fear conditioning or extinction training the second time.

RESULTS

Baseline Levels of Fear

Statistical analyses revealed that there were no group differences in BL startle during the final test in Experiments 1, 2A, or 2B (largest $F(1, 38) = 2.1$, $p > 0.05$). Likewise, in Experiment 3, there were no significant group differences in pre-CS freezing (largest $F(1, 27) = 1.04$, $p > 0.05$).

Does DCS Facilitate Extinction both the First and Second Time Around?

Experiment 1 examined the effect of DCS administration prior to extinction training using either 15 or 30 extinction trials. Comparison of the post-extinction test data from day 4 revealed that regardless of drug administered, rats given 30 extinction trials showed less FPS compared with rats given 15 extinction trials (Figure 1). Further, regardless of the number of extinction trials, rats injected with DCS showed lower levels of FPS compared with rats injected with saline. A two-way ANOVA confirmed this description of the results and revealed a significant effect of drug ($F(1, 38) = 6.69$, $p < 0.05$) and of extinction trials ($F(1, 38) = 8.62$, $p < 0.01$); the drug-by-extinction trials interaction was not significant, $F < 1$.

Experiment 1 thus replicated previous studies showing that pre-extinction training administration of DCS facilitates extinction relative to saline control rats using FPS as a measure of fear (eg, Walker *et al*, 2002). To test the hypothesis that the mechanisms involved in initial extinction may be different from those involved in extinction the second time around (re-extinction) Experiment 2A examined the role of NMDA in re-extinction.

The final test data (day 9) from Experiment 2A show that non-retrained animals had lower levels of fear compared with retrained animals (Figure 2). This indicates that the observed increase in fear is due to re-pairing the CS and US rather than spontaneous recovery. Additionally, animals that were retrained and given an injection of DCS before re-extinction did not exhibit lower levels of fear than did saline control rats. That is, DCS did not appear to facilitate re-extinction of learned fear. This description of the results was confirmed by statistical analysis. Specifically, a two-way ANOVA revealed a significant effect of retraining ($F(1, 20) = 12.47$, $p < 0.01$), but no effect of drug and no drug-by-retraining interaction, both $F < 1$.

Even though the results suggest that DCS did not facilitate re-extinction, it is possible that rats did not receive enough re-extinction. Therefore, in Experiment 2B, we reduced the amount of retraining and increased the amount of re-extinction. The test data from the final day of Experiment 2B show that rats in the DCS condition exhibited levels of FPS comparable to that seen in the rats in the saline condition (Figure 2). That is, as in Experiment 2A, DCS did not facilitate re-extinction. This description of the results

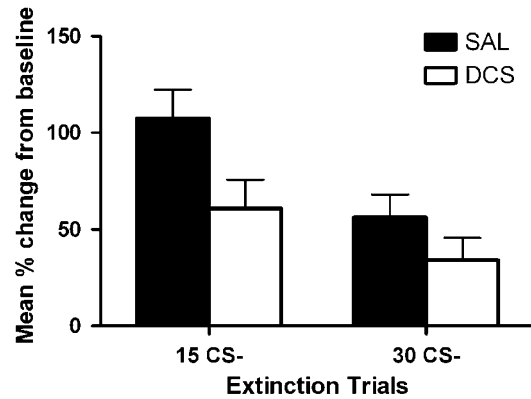


Figure 1 DCS facilitates extinction of learned fear. Mean (\pm SEM) percentage (%) change from BL startle responses at final test in Experiment 1. Rats were injected with saline or DCS before extinction training (15 or 30 CS-alone presentations). Rats were in one of four groups: 15 CS-SAL ($n = 8$), 15 CS-DCS ($n = 8$), 30 CS-SAL ($n = 13$), or 30 CS-DCS ($n = 13$).

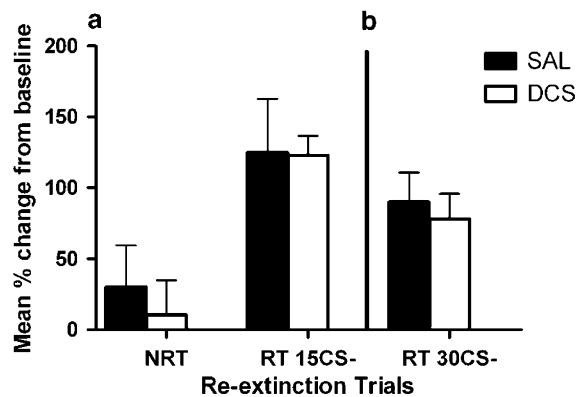


Figure 2 DCS does not facilitate re-extinction of learned fear. (a) Mean (\pm SEM) percentage (%) change from BL startle responses at final test in Experiment 2A. Rats were retrained (RT) or non-retrained (NRT) and injected with saline or DCS prior to re-extinction training (15 CS-alone presentations). Rats were in one of four groups: NRT-SAL ($n = 4$), NRT-DCS ($n = 4$), RT-SAL ($n = 8$), or RT-DCS ($n = 8$). (b) Mean (\pm SEM) percentage (%) change from BL startle responses at final test in Experiment 2B. Rats were retrained (RT) and injected with saline ($n = 13$) or DCS ($n = 13$) prior to re-extinction training (30 CS-alone presentations).

was confirmed by statistical analysis. Specifically, one-way ANOVA revealed no significant difference between DCS and saline rats, $F < 1$.

This result replicates Experiment 2A and demonstrated that even with more re-extinction training (resulting in lower levels of fear at final test than in Experiment 2A), DCS did not facilitate re-extinction. Taken together, Experiments 2A and 2B provide evidence that re-extinction involves a different process than initial extinction. Specifically, these results imply that re-extinction is NMDA-independent.

Does DCS Facilitate Extinction the Second Time Around if a Different CS is Extinguished?

Experiments 2A and 2B clearly show that DCS does not facilitate re-extinction of learned fear, measured using FPS. There are a number of interesting questions that follow from this finding. One question, in particular, is

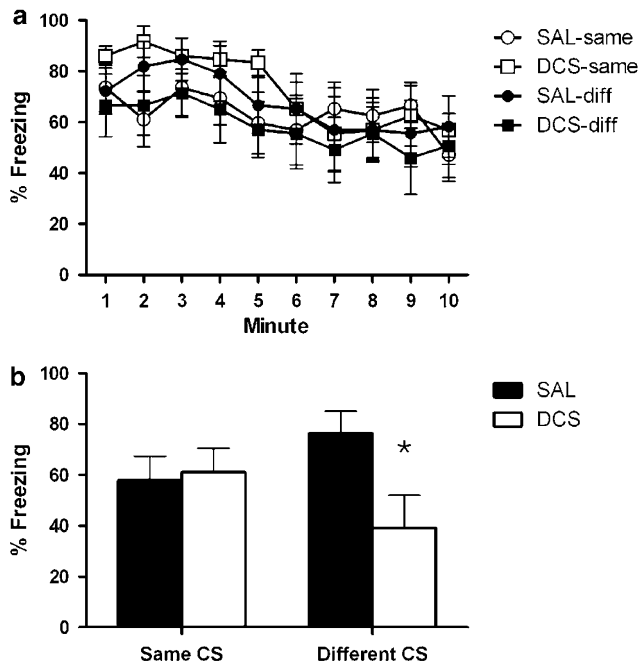


Figure 3 DCS facilitates extinction the second time around when rats are retrained with a different CS. Rats were retrained with either the same CS or different CS as initial extinction and then received an injection of saline or DCS before re-extinction training for that CS. Rats were in one of four conditions: same CS-SAL ($n = 8$), same CS-DCS ($n = 8$), different CS-SAL ($n = 8$), different CS-DCS ($n = 7$). (a) Mean (\pm SEM) freezing of rats in response to re-extinction CS during each minute of extinction training. (b) Mean (\pm SEM) freezing in response to the CS during test. *Indicates a significant difference to the SAL group being extinguished to that CS.

whether the switch from NMDA-dependent extinction to NMDA-independent re-extinction occurs by virtue of a previous training and extinction experience with any CS or whether this switch is cue specific. In other words, is re-extinction always NMDA-independent or would the effects of a second extinction session be facilitated by DCS if the retrained stimulus was a new stimulus that had not been extinguished previously? Experiment 3 aimed to address this issue as well as replicate the results of Experiments 2A and 2B using freezing as an index of fear rather than FPS. Using freezing as a measure of fear served to increase the generalizability of our findings to other fear responses and also allowed us to measure within-session re-extinction. This is important as it has been shown that DCS only facilitates extinction when within-session extinction is observed (Weber *et al*, 2007). Typically, within-session extinction measures are not taken in FPS studies because that would involve the repeated presentations of the loud, aversive startle-eliciting noise on those trials (see Walker *et al*, 2002).

The within-session re-extinction data from Experiment 3 shows that regardless of drug administered or the CS being extinguished (CS1 or CS2), all groups exhibited high levels of fear during the first minute of re-extinction that decreased to lower levels of fear by the last minute of re-extinction (Figure 3a). A three-way ANOVA confirmed this description of the results and revealed a significant effect of block ($F(1, 27) = 25.00$, $p < 0.001$), but no effect of drug, CS, or drug-by-CS interaction, $F_s < 1.0$.

Although there were no group differences in within-session re-extinction, differences were apparent the following day when rats were tested for retention of extinction learning. Specifically, rats re-extinguished to the same CS as initial extinction exhibited comparable levels of fear regardless of the drug administered prior to re-extinction. In contrast, rats extinguished for the second time with a CS different from initial extinction showed an effect of DCS. That is, rats injected with DCS prior to re-extinction training exhibited lower levels of freezing at test than rats injected with saline prior to re-extinction training (Figure 3b). This description of the results was confirmed by statistical analysis. A two-way ANOVA revealed no significant effect of drug ($F(1, 27) = 3.27$, $p > 0.05$), or CS, $F < 1.0$. However, there was a significant drug-by-CS interaction ($F(1, 27) = 4.64$, $p < 0.05$). *Post hoc* comparisons with Tukey's HSD procedure showed that this interaction was due to a significant difference between DCS and saline rats in the different CS condition ($p < 0.05$) but not in the same CS condition ($p > 0.05$).

The results of this experiment demonstrated that re-extinction is not facilitated by DCS as assessed with freezing, thereby replicating Experiments 2A and 2B. Further, this experiment shows that extinction the second time around may be facilitated by DCS when the CS is a new stimulus that has not previously been extinguished. In other words, just because fear conditioning and extinction training have previously occurred does not mean that extinction the second time is NMDA-independent. Rather, whether extinction is NMDA-independent or NMDA-dependent is determined by the history of the CS being extinguished. That is, extinction the second time around is only NMDA-independent with a CS that has previously been extinguished.

DISCUSSION

This series of experiments reveal a number of findings not predicted by current theories of extinction. Specifically, in contrast to initial extinction (Experiment 1), re-extinction is not facilitated by DCS (Experiments 2A, 2B, and 3). Therefore, re-extinction appears to be an NMDA-independent process. However, this switch from NMDA-dependent to NMDA-independent extinction is stimulus specific (Experiment 3). That is, the first extinction for any given CS is an NMDA-dependent process even if an animal has previously received fear conditioning and extinction for a different CS.

While these findings are novel, they converge with studies previously mentioned showing that learning and relearning may rely on different neurobiological processes (Roesler *et al*, 1998; Sanders and Fanselow, 2003; Cammarota *et al*, 2004). The current series of experiments extend this previous research by demonstrating that extinction and re-extinction also rely on different neurobiological processes. Specifically, just as relearning is NMDA-independent (Roesler *et al*, 1998; Sanders and Fanselow, 2003; Cammarota *et al*, 2004), re-extinction appears to be NMDA-independent.

Until the current series of experiments, the issue of whether extinction relies on the same neurobiological

mechanisms the first and second time around had not been addressed. In particular, NMDA receptors are considered vital for both the acquisition and extinction of fear; however, the current results question the involvement of NMDA in extinction under all circumstances. Such a finding has interesting implications for theoretical models of extinction. Specifically, the involvement of NMDA receptors in extinction has been taken as evidence that extinction is a new learning process. Therefore, under circumstances where extinction is NMDA-independent (ie, re-extinction), extinction may be dominated by processes other than new learning. This result draws further attention to the inadequacy of a single mechanism to explain extinction and the need for a hybrid model that incorporates new learning, erasure, and nonassociative processes (see Myers and Davis, 2007).

In terms of neurobiological models of extinction, the current results suggest that neurotransmitter systems other than glutamate (NMDA) must be primarily mediating re-extinction. Further, perhaps the neural circuitry involved in re-extinction is fundamentally different from that involved in initial extinction. Recent neural models of extinction propose that interactions between the amygdala, hippocampus, and ventromedial prefrontal cortex (vmPFC) are involved in extinction (Corcoran and Quirk, 2007; Myers and Davis, 2007). Studies suggest that neural plasticity associated with fear learning and the acquisition of extinction occurs in the amygdala, which is also the site of fear memory storage (Corcoran and Quirk, 2007; Myers and Davis, 2007; Sotres-Bayon *et al*, 2007). The vmPFC is thought to be involved in the consolidation of extinction memories (Hugues *et al*, 2004, 2006; Burgos-Robles *et al*, 2007), and interactions between the hippocampus and vmPFC are considered essential in inhibiting the amygdala fear response during an extinction retention test (Corcoran and Quirk, 2007). Although this neural model has not been explicitly examined during re-extinction, two recent studies suggest that the neural circuitry involved in re-extinction is different from that involved in initial extinction (Morgan *et al*, 2003; Weber M, Westbrook RF, Carrive P, Richardson R, unpublished observations). First, temporary inactivation of the amygdala prior to extinction training impaired both within-session extinction and retention of extinction; however, it did not impair re-extinction (Weber M, Westbrook RF, Carrive P, Richardson R, unpublished observations; also see Kim and Richardson, 2008). Second, it has been demonstrated that bilateral mPFC lesions caused significantly more impairment during re-extinction than initial extinction (Morgan *et al*, 2003). More specifically, rats with mPFC lesions took significantly longer to show a reduction in conditioned responding during re-extinction compared with initial extinction. In other words, mPFC lesions during re-extinction caused greater resistance to extinction compared with mPFC lesions during initial extinction. Collectively, these studies suggest that the amygdala is not required for re-extinction, whereas the mPFC may play a more prominent role in re-extinction than initial extinction. That is, initial extinction involves the formation of a new extinction memory that requires neural plasticity in the amygdala and vmPFC and activity in the vmPFC–hippocampal circuit during retrieval of the extinction memory. In contrast, if re-extinction involves retrieval

of an initial extinction memory, this process would not require neural plasticity in the amygdala (as the extinction memory is already present); however, the re-extinction process would require the vmPFC–hippocampal circuit.

Finally, the current findings also have important clinical implications given that extinction is a useful animal model for exposure therapies. Indeed, translation from preclinical to clinical trials using DCS as an adjunct to exposure therapy has demonstrated the practical utility of such preclinical work (Davis *et al*, 2006; Hofmann, 2007). Moreover, considerable evidence that the neural circuitry involved in extinction in rodents is very similar to the circuits involved in emotion regulation in humans further confirms the value of preclinical studies (Phelps *et al*, 2004; Quirk and Beer, 2006). These examples indicate that understanding the mechanisms involved in extinction, and, in particular, understanding what manipulations facilitate extinction serve to improve therapies for anxiety disorders. Although exposure-based therapies are highly efficacious (Foa, 2006), relapse following successful treatment is a common problem (Rachman, 1989). Surprisingly, extinction or exposure therapy after relapse has received limited attention in the empirical literature. In light of the current findings, it seems that treating individuals after relapse may be fundamentally different from treatment the first time. More specifically, the results suggest that extinction is not always mediated by the same neurobiological mechanisms. On the basis of this, it may be the case that exposure therapy after relapse may be mediated by different processes compared with exposure therapy the first time. In particular, while studies show that DCS as an adjunct to exposure therapy facilitates treatment (Ressler *et al*, 2004; Hofmann *et al*, 2006; Kushner *et al*, 2007; Guastella *et al*, in press), the effectiveness of such treatments following relapse remains to be tested.

In conclusion, the current study demonstrates that re-extinction is an NMDA-independent process. The finding that DCS does not facilitate re-extinction was seen using both FPS and freezing as a measure of fear across a number of experiments. These findings have implications for theoretical and neural models of extinction. Specifically, they suggest that a hybrid model is required to explain extinction, and they question the idea that NMDA receptor action is a requirement for extinction under all circumstances. The results also have potential clinical importance for the treatment of anxiety disorders following relapse.

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DISCLOSURE/CONFLICT OF INTEREST

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