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# D-Cycloserine facilitates context-specific fear extinction learning

Mark E. Bouton *, Drina Vurbic, Amanda M. Woods

*Department of Psychology, University of Vermont, 2 Colchester Avenue, Burlington, VT 05405-0134, USA*

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ABSTRACT

D-Cycloserine (DCS) may facilitate fear extinction learning, but the behavioral consequences and mechanisms behind this effect are not well understood at present. In this paper, we re-analyze data from previously reported null result experiments and find that rats showing above-median extinction learning during DCS treatment benefited from the drug, whereas rats showing below-median (and in this case little) extinction learning did not. Two additional experiments found that DCS facilitated extinction learning when specifically combined with a moderate, but not a small, number of extinction trials. DCS thus facilitates extinction learning only if the behavioral procedure first engages the extinction learning process. The benefits of the drug, however, were specific to the context in which extinction was learned—i.e., DCS did not prevent or influence the renewal of fear observed when the extinguished cue was tested in the original conditioning context.



## 1. Introduction

When a conditional stimulus (CS, e.g., a tone) has been associated with an aversive unconditional stimulus (US, e.g., footshock), presentation of the CS will evoke fear. This form of conditioning may play a role in the etiology of many anxiety disorders (e.g., Barlow, 2002; Bouton, Mineka, & Barlow, 2001; Mineka & Zinbarg, 2006). Importantly, conditioned fear can be reduced by repeated presentation of the CS without the US. This phenomenon, known as *extinction*, is widely used as a tool in therapy. However, although extinction seems to eliminate fear, it does not reflect an erasure of the original fear learning (e.g., Bouton, 2004; Bouton, Westbrook, Corcoran, & Maren, 2006; Rescorla, 2001). For example, fear of the CS returns if the context is changed after extinction, a phenomenon known as the *renewal effect* (e.g., Bouton & King, 1983). This result, among others, suggests that extinction depends at least partly on new learning that depends on the context for retrieval. This principle has a number of implications for the success of therapies that rely on extinction for their beneficial effects (e.g., Bouton, 2002).

If extinction involves new learning, then the loss of fear should be enhanced if the organism is given a drug that can facilitate learning. Consistent with this view, administration of D-cycloserine (DCS), a partial agonist of the NMDA receptor involved in long-term potentiation (a cellular model of learning), facilitates extinction (e.g., Ledgerwood, Richardson, & Cranney, 2003; Walker, Ressler, Lu, & Davis, 2002). Rats given DCS with a small number of extinction trials show less fear than control subjects that receive the same number of trials without DCS during tests of the CS conducted the next day. The results have clinical significance. In humans, DCS administration can likewise facilitate the loss of fear resulting from exposure that causes incomplete fear loss in controls (acrophobia: Ressler et al., 2004; social phobia: Guastella et al., 2008; Hofmann et al., 2006; obsessive–compulsive disorder: Kushner et al., 2007).

Although DCS can lead to faster extinction, we currently know little about the boundary conditions of its effect. What are the best conditions for delivering DCS to facilitate extinction learning? And does extinction with DCS cause a more permanent, or fundamentally different, form of extinction learning? Woods and Bouton (2006) found that although DCS facilitated fear loss in extinction, it did not weaken the renewal effect. Rats received CS–shock pairings in one context and then four extinction trials (CS—no shock presentations) in a second context. The four extinction trials were preceded by an injection of saline or DCS (15 or 30 mg/kg). Subsequent tests in the context of extinction revealed that rats that had received the 30 mg/kg dose of DCS were less afraid of the CS than controls that had received extinction with saline. However, when the CS was then tested in the original conditioning context, the DCS group showed a substantial renewal of fear that was similar in strength to the one observed in the saline controls. Thus, although DCS facilitated extinction learning, it did not change extinction's fundamental dependence on the context.

Woods and Bouton (2006) also mentioned other results suggesting further boundary conditions for the effects of DCS. In two unpublished experiments, DCS had no demonstrable effect on extinction learning. Such null results are consistent with reports

* Corresponding author. Fax: +1 802 656 8783.
*E-mail address:* Mark.Bouton@uvm.edu (M.E. Bouton).

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in humans suggesting that DCS does not necessarily facilitate the extinction of spider fear (Guastella, Dadds, Lovibond, Mitchell, & Richardson, 2007), electrodermal conditioning (Guastella, Lovibond, Dadds, Mitchell, & Richardson, 2007), or at least one example of obsessive–compulsive disorder (Storch et al., 2007). Such findings suggest there is a need to isolate the variables that modulate DCS's effects on extinction learning.

One clue is provided by two recent reports. Weber, Hart, and Richardson (2007) examined the effects of DCS on the extinction of fear to an odor CS. Consistent with the research just described, DCS did not always facilitate the extinction of fear. However, the authors noted that not all subjects showed evidence of learning extinction during the session when the drug was given. When they separated subjects that had learned some extinction from those that had not, only the rats that had demonstrably learned some extinction benefited from the DCS. Unfortunately, the amount of extinction learning was not manipulated experimentally, and DCS's effectiveness with rats that had learned some extinction might therefore be explained by an unidentified third variable that also distinguished rats that had learned some extinction from those that had not. In contrast, Lee, Milton, and Everitt (2006) manipulated the amount of extinction learning experimentally. After fear conditioning with an auditory CS, they found that (1) when DCS was combined with minimal exposure to the CS, there was an increase in fear during a subsequent test, and (2) when DCS was combined with more extinction exposure to the CS, there was less subsequent fear. They suggested that DCS enhanced memory "reconsolidation" (Nader, Schafe, & LeDoux, 2000) when it was combined with minimal CS exposure and enhanced extinction when it was combined with more. The results suggest that the amount of non-reinforced exposure to the CS, and extinction learning, may be an important factor in predicting the effects of DCS. But the experimental design did not guarantee that the specific combination of drug and CS re-exposure produces the effects, because the control groups that had received equivalent drug exposure had no re-exposure to the CS between conditioning and testing. Isolation of a role for the drug + CS exposure combination would require a control that receives equal but separate exposure to both the drug and the CS.

The research presented in this paper further extends the analysis of the role of extinction learning on the effectiveness of DCS. We began by reanalyzing the null results of Woods and Bouton (2006) to ask whether the effectiveness of DCS was correlated with the success of extinction learning while the rat was under the influence of the drug. We then asked experimentally whether more extinction learning allows facilitation of extinction by DCS using an experimental design that uniquely isolated the importance of the combination of DCS and CS exposure. Having established a role for DCS, we also further characterized its effect by asking whether it reduces the context-dependence of extinction, or merely enhances the rate of normal extinction learning, which can be highly dependent on the context.

## 2. Experiment 1

In the first experiment, we fully report the two null experiments mentioned by Woods and Bouton (2006) and also ask whether there was a correlation between extinction learning during the drug session and the effectiveness of the drug. Woods and Bouton (2006) used the conditioned suppression method, in which fear of a CS is indexed by the CS's ability to suppress an ongoing operant lever pressing baseline reinforced by food. This method has a long history in the study of fear conditioning (e.g., Estes & Skinner, 1941; Kamin, 1969; Rescorla, 1968) and has been use extensively in research on fear extinction (e.g., Bouton, 2004; Bouton & King, 1983).

### 2.1. Method

#### 2.1.1. Subjects

The two experiments involved a total of 48 female Wistar rats (Charles River, Quebec, Canada), 75- to 90-days-old at the start of the experiment. The rats were individually housed and food-deprived to 80% of their baseline body weights. Water was available ad lib, and the experiments were run on consecutive days during the light portion of a 16:8 h light-dark cycle.

#### 2.1.2. Apparatus

There were two counterbalanced sets of four Skinner boxes located in separate rooms of the laboratory. Boxes from both sets measured 31.75 × 24.13 × 29.21 cm (l × w × h) and were housed in sound-attenuation chambers. The front and back walls were aluminum; the side walls and ceiling were clear acrylic plastic. There was a 5.08 × 5.08 cm recessed food cup centered in the front wall near floor-level. A 4.8-cm stainless steel operant lever was located to the left of the food cup, 6.2 cm above the floor. Ventilation fans provided background noise of 60 dB, and illumination was provided by two 7.5-W incandescent bulbs on the ceiling of the sound-attenuation chamber. The light-off CS (60 s) was created by terminating the houselights. The US was a 0.5 s, scrambled 1-mA shock provided by Med Associates shock sources. Lever pressing was reinforced with 45-mg food pellets.

In one set of boxes, the floor consisted of 0.48-cm diameter stainless steel grids spaced 3.81 cm and mounted parallel to the front wall. The ceiling and a side wall had black horizontal stripes (3.81 cm wide). A dish containing a 2% anise solution (McCormick) was placed outside each box to provide an odor. In the other set of boxes, the floor consisted of alternating stainless steel grids with different diameters (0.48 and 1.27 cm), spaced 1.59 cm. The ceiling and left sidewall were covered with dark dots (1.9 cm in diameter). A dish containing 5 ml of a 4% coconut solution (McCormick) was placed outside each box to provide odor.

#### 2.1.3. Procedure

The procedure was the same as that described by Woods and Bouton (2006) through baseline training, fear conditioning, extinction, and testing.

*2.1.3.1. Baseline training and conditioning.* The rats were first trained to lever press on a variable-interval (VI) 90-s reinforcement schedule. There were eight daily 60-min sessions; half occurred in Context A (Days 1, 2, 3, 7) and the other half occurred in Context B (Days 4, 5, 6, 8). On Day 9, there was one 84-min session in which the rats received fear conditioning in Context A. Each of 12 presentations of the CS terminated in the onset of the US. The interval between trials (ITI) averaged 6 min. On Day 10, there was one 60-min baseline-recovery session in Context B in which the rats merely lever pressed on the VI-90 s schedule. The two groups of rats (saline or DCS 30 mg/kg) were then matched on their baseline rates.

*2.1.3.2. Extinction and testing.* On Day 11, there was one 64-min session containing four CS-alone (extinction) trials in Context B. The ITI averaged 15 min. DCS or saline was administered 15 min prior to the session. On Days 12 and 13, there were two similar test sessions (one per day) in Context B, each involving four more extinction trials with the CS. The Day 11 session, when the drug was administered, is referred to as the "drug session", while the other two sessions are referred to as "test sessions".

*2.1.3.3. Drug administration.* D-Cycloserine (Sigma–Aldrich, St. Louis, MO) was mixed (immediately prior to use) with 0.9% physiological saline and injected subcutaneously in a volume of