The effect of D-cycloserine on immediate vs. delayed extinction of learned fear

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We compared the effect of D-cycloserine (DCS) on immediate (10 min after conditioning) and delayed (24 h after conditioning) extinction of learned fear in rats. DCS facilitated both immediate and delayed extinction when the drug was administered after extinction training. However, DCS did not facilitate immediate extinction when administered prior to extinction training (i.e., when the interval between drug administration and shock was reduced). In addition, administering five, but not two, shocks prior to extinction training disrupted the facilitating effects of DCS on delayed extinction. These results suggest that aversive experiences prior to DCS administration can prevent it from facilitating extinction.
described previously (see Werner-Seidler and Richardson 2007). Briefly, fear conditioning (context A) consisted of five CS–US pairings (2 min intertrial interval [ITI]), extinction training (context B) consisted of six CS-alone presentations (2 min each; 2 min ITI), and test (context B) consisted of one 2-min CS presentation. An ABB design was used to reduce pre-CS freezing during extinction training and test. Rats were injected with either saline or DCS (15 mg/kg; Sigma) prior to or following the extinction training session (for further details, see Langton and Richardson 2008).

The measure of fear used was freezing, which was defined and scored as described previously (see Langton and Richardson 2008, 2009); 30% of the test data was scored by a second observer blind to the study details (rs > 0.90). Data were analyzed using independent sample t-tests and analysis of variance (ANOVA).

Post-hoc tests were done with Tukey’s honestly significant difference (HSD) procedure. There were no group differences in pre-CS freezing at the start of extinction or at test across the three experiments (largest F(1,38) = 2.71, P = 0.11).

Experiment 1 compared the effect of DCS on extinction that occurred either immediately (10 min) after conditioning or following a delay (24 h). Either 10 min or 24 h after fear conditioning rats received extinction training, and were subsequently injected with DCS or saline and tested for fear of the CS 24 h later. Therefore, this experiment examined the effect of DCS on long-term extinction retention (as opposed to within-session extinction) following immediate vs. delayed extinction training. All groups showed evidence of within-session extinction (Fig. 1A) with statistical analysis revealing a significant effect of trial, F(1,38) = 5.22, P < 0.05, but no significant interaction effects (F < 1) or other main effects reached significance. At test the following day, regardless of whether extinction occurred immediately after conditioning or after a 24 h delay, DCS-treated rats exhibited less fear than did saline-treated rats (Fig. 1B): analysis of the test data revealed a significant effect of drug, F(1,38) = 5.72, P < 0.05, but no effect of time or drug-by-time interaction (F < 1).

Experiment 1 showed that DCS facilitates both immediate and delayed extinction when DCS is administered following extinction training. Specifically, DCS facilitated immediate extinction when the interval between fear conditioning and drug administration was 35 min. To further explore the effect of DCS on immediate extinction, we compared the effect of administering DCS either prior to or following immediate extinction training. In Experiment 2, all rats were extinguished 10 min after fear conditioning and tested for CS-elicited freezing the following day. Rats were injected with DCS or saline either immediately before or after the extinction session. The saline-prior and saline-after groups were collapsed (analysis revealed no significant difference between the groups; F(1,18) = 0.01, P = 0.99) such that there were three groups—Saline, DCS-prior, and DCS-after. All groups showed evidence of within-session extinction (Fig. 2A); statistical analysis revealed a significant effect of trial, F(1,23) = 67.31, P < 0.001, but no effect of group or trial-by-group interaction (F < 1). At test the following day, rats injected with DCS after immediate extinction exhibited less freezing compared to saline-treated animals and rats injected with DCS prior to immediate extinction training (Fig. 2B). A one-way ANOVA confirmed this and revealed a significant effect of group (F(2,23) = 10.14, P < 0.05). Post-hoc tests showed that this effect was due to both the saline and DCS-prior groups being significantly different to the DCS-after groups (F < 0.05); there was no significant difference between the saline and DCS-prior groups (P > 0.75).

Experiment 2 showed that DCS did not facilitate immediate extinction when it was administered 10 min following the last CS–US pairing, but did facilitate immediate extinction when it was administered 35 min after the last CS–US pairing. This suggests that the interval between fear conditioning and DCS administration may influence the effect of DCS on extinction. In order to examine whether recent fear conditioning (i.e., associative learning) is the crucial factor is reducing the efficacy of DCS on extinction or whether any recent aversive experience can prevent DCS from facilitating extinction, Experiment 3 examined the effect of DCS on delayed extinction that was preceded by foot-shock. Fear conditioning (context A) occurred on day 1 and on day 2 rats were returned to context A and given either two or five shocks (1 sec, 0.6 mA with 30 sec ITI); 10 min later they were injected with DCS or saline and received extinction training in context B and were tested for fear of the CS on day 3.

Regardless of drug or number of shocks delivered, all rats showed evidence of within-session extinction (Fig. 3A). Statistical analysis confirmed this description of the data and revealed a significant effect of trial, F(1,38) = 52.17, P < 0.001, but no effect of drug (F < 1), and no significant interaction effects (largest F(1,38) = 2.69, P = 0.11). At test the following day, both DCS- and saline-treated rats in the five-shock condition showed comparable levels of freezing. In contrast, in the two-shock condition DCS-treated rats appeared to show less fear than saline-treated rats (Fig. 3B). Statistical analysis of the test data revealed a significant effect of drug, F(1,38) = 8.69, P < 0.05, no effect of shock (F < 1), and a drug-by-shock interaction, F(1,38) = 5.91, P = 0.05. Post-hoc tests showed that the interaction was due to a significant difference between saline and DCS rats in the two-shock condition (P < 0.05), but not in the five-shock condition (P = 0.98). No other post-hoc comparisons were significant. These results show that DCS facilitates delayed extinction that is preceded by two, but not five, footshocks.

Taken together, these results demonstrate that DCS facilitates both immediate and delayed extinction. However, if there

Figure 1. Extinction training and test data from Experiment 1. This experiment demonstrates that DCS facilitates extinction that occurs immediately (10 min) or following a delay (24 h) after fear conditioning (when DCS is injected after the extinction training session). This is shown by lower levels of fear at test in rats injected with DCS compared to saline. Rats were in one of four groups: Immed-SAL (n = 10), Immed-DCS (n = 10), Delay-SAL (n = 10), or Delay-DCS (n = 12). (A) Mean (+ SEM) freezing of rats during trial 1 and trial 6 of extinction training. (B) Mean (+ SEM) freezing in response to the CS during test. * Indicates a significant difference.

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is a short interval (~10 min) between DCS administration and strong stimulation (fear conditioning or five shock presentations), then DCS facilitation of extinction is disrupted. Importantly, neither fear conditioning nor shock presentations prior to extinction training disrupted within-session extinction, which is an important requirement for DCS facilitation effects (e.g., Weber et al. 2007; Bouton et al. 2008). That is, the recent experience of fear conditioning/shock did not prevent within-session extinction, but it did prevent the DCS enhancement of the consolidation of this learning.

The current research represents an investigation of the neurotransmitter systems involved in immediate extinction and suggests that immediate extinction, like delayed extinction, is NMDAr dependent unless DCS administration occurs soon after (~10 min) fear conditioning or footshock presentations. This result is similar to the results reported by Cain et al. (2005) who found that the interval between conditioning and nifedipine administration influenced the effect of the drug on extinction. When there was no interval, the drug had no effect (immediate group) and when the interval was largest, the drug produced the greatest impairment (3- and 24-h groups). Likewise, in the current experiments when the interval between fear conditioning/shock and drug administration was short (~10 min) DCS did not facilitate extinction, but when the interval was longer (~35 min or 24 h) DCS did facilitate extinction.

The finding that DCS does not facilitate extinction when closely preceded by strong stimulation (such as the shock used during fear conditioning) is consistent with the relative lack of evidence showing that DCS facilitates fear conditioning in comparison to other types of learning. For example, DCS has been shown to facilitate a number of associative learning tasks including conditioned taste aversion in rats (Land and Riccio 1997) and trace eyeblink conditioning in rabbits using an air-puff US (Thompson et al. 1992; Thompson and Disterhoft 1997). DCS has also been found to facilitate reconsolidation of a fear conditioning memory (Lee et al. 2006), passive avoidance learning (Monahan et al. 1989; Land and Riccio 1997; but see Tomilenko and Dubrovina 2007), and a number of spatial learning tasks (Monahan et al. 1989; Baxter et al. 1994; Quartermain et al. 1994; Lelong et al. 2001). Additionally, compared to the large body of research showing DCS enhancement effects on extinction (see Norberg et al. [2008] for a meta-analysis) there are only a handful of studies showing that DCS enhances CS or contextual fear conditioning (Silvestri and Root 2008; Kalisch et al. 2009; Waddell et al. 2010; but see Yamamoto et al. 2008).

Based on the relative lack of evidence that DCS facilitates fear conditioning compared to extinction, Davis et al. (2005, 2006) proposed that repeated presentations of aversive stimuli (that occur during fear conditioning, for example) may increase endogenous levels of glycine and D-serine such that the application of DCS has no benefit (i.e., does not enhance learning). Specifically, it has been suggested that when the glycine site of the NMDAr is saturated, the capacity for positive modulation of the NMDAr is reduced, which makes it unlikely that application of a drug such as DCS will be able to enhance learning and/or memory consolidation (Davis et al. 2005). The impact of increased endogenous glycine on the effects of NMDAr agonists has been demonstrated in two recent studies. For example, Li et al. (2009) used whole cell voltage-clamp recordings to explore the effects of strong and weak synaptic inputs on the action of positive modulators of the glycine site of the NMDAr in rat hippocampal slices. They found that strong synaptic stimulation increased endogenous glycine levels and prevented NMDAr agonists from positively modulating NMDAr-mediated responses (i.e., excitatory postsynaptic currents [EPSCs]), whereas NMDAr agonists potentiated EPSCs when weak synaptic stimulation was applied.
Similarly, Zhang et al. (2008) examined the effect of the glycine site partial agonist GLYX-13 on long-term potentiation (LTP), long-term depression (LTD), and NMDAR-mediated ESPCs in hippocampal slices. They found that GLYX-13 modulation of LTP, LTD, and ESPCs was prevented when D-serine was applied to the hippocampal slices at saturating concentrations. These studies demonstrate that drugs working on the glycine site of the NMDAR are unable to modulate the synaptic plasticity that underlies learning and memory when the glycine site of the NMDAR is saturated. Importantly, the glycine-site of the NMDAR is not saturated under baseline conditions or even following weak synaptic inputs in physiological studies (Bergeron et al. 1998; Li et al. 2009). Therefore, although speculative, a relationship between synaptic inputs/stimulation and levels of endogenous glycine can explain why DCS facilitated extinction following two shocks (i.e., weak stimuli) but not five shocks (i.e., strong stimuli). Further, the current results suggest that any increase in endogenous glycine following repeated shock presentations must be short-lived, as DCS was found to facilitate extinction when delivered 35 min (but not 10 min) following fear conditioning. Studies measuring neural levels of endogenous glycine following aversive stimulation are required to provide direct evidence for the hypothesis that DCS does not facilitate learning when the procedure involves repeated shock presentations because of short-lived saturation at the glycine-site of the NMDAR.

In summary, the current findings contribute to our understanding of the role of aversive stimulation on NMDAR modulating agents, such as DCS, and also contribute to the literature on the neurotransmitter systems involved in immediate vs. delayed extinction. Additionally, determining situations that will influence the effect of DCS on extinction is clinically important given research demonstrating that DCS effectively augments exposure therapy for a number of anxiety disorders (see Norberg et al. 2005; Werner-Seidler and Richardson 2007). The current results show that DCS does not facilitate extinction if it is administered soon after a stressor such as shock or fear conditioning (a clinical analogy to this may be trauma or a panic attack), suggesting that DCS may be an inappropriate adjunct for early intervention treatments following trauma. Understanding the circumstances when pharmacological adjuncts to exposure therapy, such as DCS, may be ineffective will assist clinicians and researchers to refine treatment protocols for anxiety disorders in the future.

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