D-Cycloserine Fails to Facilitate Extinction of a Conditioned Taste Aversion and Potentiates Spontaneous Recovery

G. Andrew Mickley, Gina. N. Wilson, Jennifer Remus, and Orion Biesan

Neuroscience Program and Department of Psychology, Baldwin-Wallace College, Berea, OH 44017

Abstract

D-cycloserine (DCS), the glutamate NMDA receptor partial agonist, has been reported to facilitate the extinction of learned fears acquired in both naturalistic and laboratory settings. The current study extended this literature by evaluating the ability of DCS to modulate the extinction (EXT) and spontaneous recovery (SR) of a conditioned taste aversion (CTA). Twenty-three hour fluid-deprived Sprague-Dawley rats acquired a strong CTA following 3 CS+US pairings [0.3% oral saccharin (SAC) and 81 mg/kg (i.p.) Lithium Chloride (LiCl)]. In different groups of rats we then employed 2 different EXT paradigms: (1) CS-only (CSO) in which SAC was presented every-other day, or (2) Explicitly Unpaired (EU) in which both SAC and LiCl were presented but on alternate days. Our previous studies indicated that SR of a CTA emerges following CSO EXT but the EU-EXT paradigm causes a suppression of SR (Mickley et al., 2009). DCS (15 mg/kg, i.p.) was administered immediately after daily liquid presentations (SAC or water, alternate days) during the EXT training period (Mean ± SEM = 36.83 ± 1.86 days). Once rats met our criterion for asymptotic EXT (90% reacceptance of the CS) they entered a 30-day latency period during which they received only water for 1 hr/day. The next day, a final opportunity to drink SAC was provided (SR test). In a replication of our previous work, rats that went through the EU-EXT procedure achieved asymptotic extinction more quickly than did the CSO rats and did not exhibit a SR of the CTA. However, DCS failed to shorten the time required to reach asymptotic EXT when either method was employed. Both CSO and EU rats that received DCS treatments exhibited a SR of the CTA - suggesting that the drug not only failed to facilitate EXT but may also enhance the SR of this defensive reaction to a learned fear. Additional control experiments confirmed that an acute injection of 15 mg/kg (i.p.) DCS does not eliminate the rat's ability to discriminate between 0.3% and 0.6% SAC nor does it act as an effective US. Therefore, the drug effects we report here are unlikely the result of changes in our animal's ability to taste or DCS's potentiation of LiCl-induced malaise. These data raise doubts about the general efficacy of chronic DCS treatments as a means to facilitate extinction of defensive reactions to conditioned fears.

Introduction

- Fears may be acquired through associations of previously neutral stimuli with painful or aversive experiences - yielding phobias or post-traumatic stress disorder.
- Fears may be reduced through various exposure therapies in which the object of fear (CS) is presented again, this time without the aversive stimuli (US), in an attempt to disassociate the CS – US connections (Foa, 2000; Basoglu, 2007).
- Fear extinction may be temporarily successful; however, spontaneous recovery (SR) and renewal of the fear (e.g. flashbacks) impede therapeutic progress (Bouton, 2002).
- Using a Conditioned Emotional Response (CER) paradigm, Thomas et al., (2005) have explored ways to manipulate the extinction process to reduce renewal. They used an explicitly unpaired (EU) extinction procedure in which subjects received both the CS and US but in a temporal relationship that did not allow for the CS-US association to be made.
- Our laboratory has been studying a different defensive reaction to a learned fear – the conditioned taste aversion (CTA) paradigm – in which a novel taste (CS) is associated with the symptoms of poisoning (US) (Mickley et al., 2004; 2005).
- The resulting aversion to, and avoidance of, the feared taste can slowly be extinguished by repeated exposure to the CS alone. However, this CS-only extinction procedure allows spontaneous recovery of the CTA (Mickley et al., 2007).
- We have recently reported that, by explicitly unpairing the CS (taste) and US (sensation of malaise) during extinction, we could speed up the extinction process and reduce spontaneous recovery of the CTA (Mickley et al., 2009).
- Drug treatments have also been proposed as a way to facilitate extinction learning.
  - D-Cycloserine (DCS), a partial NMDA agonist at the glycine site, has been shown to enhance CTA conditioning (Nunnink, Davenport, Ortega & Houpt, 2007; Davenport & Houpt, 2009) and extinction in other fear-based behavioral models (Ledgerwood, Richardson & Cranney, 2003 & 2004; Walker et al., 2002). **However, the use of DCS to modulate extinction learning has not been tested using the CTA paradigm.**
- **The current study investigated the efficacy of D-Cycloserine as a possible facilitator of CTA extinction. We tested DCS effects on rats that acquired a strong CTA and then underwent extinction through exposure to the CS only (CSO) or using the alternate extinction procedure where CS and US were explicitly unpaired (EU-EXT procedure).**

Methods

Preliminary Parametric Experiment 1: *Does DCS change the ability of rats to taste Saccharin or feel LiCl-induced malaise?*

Table 1: Experiment 1Group Nomenclature and Treatments

Group Nomenclature	N	Treatments (Measures) on Discrimination Days 1 & 3	Liquid Available on Rest Days 2 & 4	Treatments (Measures) on LiCl Test Day 5
SAL	10	SAL[1] + (SAC[2])	Water	SAL + LiCl[4] (Water)
DCS 3mg	10	DCS[3] + (SAC)	Water	DCS + LiCl (Water)
DCS 7mg	10	DCS + (SAC)	Water	DCS + LiCl (Water)
DCS 15mg	10	DCS + (SAC)	Water	DCS + LiCl (Water)

[1]SAL = physiological saline injection (0.9% NaCl dissolved in deionized water; 1ml/kg, i.p.)
[2]SAC = two-bottle SAC preference test using 0.3%SAC and 0.6%SAC [%w/v; SAC salt dissolved in deionized water to specific concentration].
[3]DCS= DCS injection (DCS dissolved in physiological saline to either 3, 7 or 15mg/ml; 1ml/kg, i.p.; dosing specified in group nomenclature)
[4](LiCl+Water) = LiCl injection (81mg/ml LiCl dissolved in physiological saline; dose = 81 mg/kg, i.p) which was immediately followed by the presentation of one water bottle.

DCS effects on saccharin discrimination and LiCl-induced malaise

- Naïve, Sprague-Dawley male and female rats were used
- Two days prior to experimental manipulations, all animals were placed on a 23-hr fluid deprivation schedule and maintained on this schedule throughout the study. Daily fluid consumption (water and/or SAC) was measured to an accuracy of 0.1g.
- On the next day animals were simultaneously offered two sipper bottles of 0.3% and 0.6% SAC for 30min on the second day and then 30min of water to prevent dehydration. This SAC exposure was meant to avert neophobia on subsequent discrimination testing days.
  - Bottle positions for all two-bottle preference testing were switched at 1, 5, and 10min into fluid presentation to force the rats to sample from both drinking tubes.
- On Days 1 and 3 of testing, animals were given an injection of DCS and 30min later presented with 0.3% and 0.6% SAC. They were then given 30min of water access.
- Days 2 and 4 were rest days on which the animals received 1 hr of water only and no injections.
- On Day 5 of the study, animals were assessed for symptoms of visceral malaise following a LiCl injection.
  - Animals were given an injection of DCS (refer to Table 1). Thirty min later they were all given an injection of LiCl (81mg/kg, i.p.) and presented with a single sipper tube of water.
  - "Lying-on-belly" (LOB) is a behavioral response, which is characterized by periods of immobility with a flattened, stretched-out posture (Meachum & Bernstein, 1990).
  - We observed the animals' activity every 2 min, for 30 min total, and recorded behaviors as either LOB or not engaging in LOB.

Experiment 2: *Does DCS facilitate extinction of a CTA or eliminate SR?*

Table 2: Experiment 2 Group Nomenclature and Treatments

Group Nomenclature	N through EXT	Conditioning		Extinction		N at SR	SR Test Solution
		Days 1, 3, 5	Days 2, 4, 6	Odd Days	Even Days		
CSO(SAL)	10	SAC[1] + LiCl[2]	Water	SAC + SAL[3]	Water + (SAL + SAL)[4]	6	SAC
CSO(DCS)	11	SAC + LiCl	Water	SAC + DCS	Water + (SAL + DCS[5])	6	SAC
EU(SAL)	9	SAC + LiCl	Water	SAC + SAL	Water + (LiCl + SAL)	6	SAC
EU(DCS)	10	SAC + LiCl	Water	SAC + DCS	Water + (LiCl + DCS)	6	SAC

[1]SAC = 0.3% SAC solution given orally and presented for 30min at a time [0.3% w/v SAC salt dissolved in deionized water].
[2]LiCl= LiCl injection (81mg/kg given at a volume of 1ml/kg, i.p.; LiCl dissolved in physiological saline)
[3]SAL = physiological saline injection (0.9% NaCl dissolved in deionized water; 1ml/kg, i.p.)
[4](SAL + SAL) = parenthetical designation indicates two injections given within 30s of each other in the order listed above. In the case of the CSO(SAL) group these animals were just given two injections of SAL. mg/kg, i.p) which was immediately followed by the presentation of one water bottle.
[5]DCS= DCS injection (15mg/kg given at a volume of 1ml/kg, i.p.; DCS dissolved in physiological saline)

DCS effects on Extinction of a CTA

CTA Acquisition

- Naïve male Sprague-Dawley rats were used.
- Two days prior to experimental manipulations, all animals were placed on a 23-hr fluid deprivation schedule and maintained on this schedule throughout the study.
- On Days 1, 3, and 5 of the study, fluid-deprived animals were given 30min access to 0.3% SAC and immediately injected with LiCl (81mg/kg, i.p.).
- Fifteen min following SAC exposure animals were given 30min access to water.
- Days 2, 4, and 6 served as rest days on which the animals were given water only and no injections.

Extinction

- Animals were given a 30min presentation of 0.3% SAC, followed 15min later by 30min access to water, every other day (odd days; refer to Table 2) throughout EXT until they reached 90% of baseline SAC consumption (designated "asymptotic" extinction; See Nolan et al, 1997).
- On odd-numbered days animals received an injection of DCS (15mg/kg) or SAL following SAC exposure.
- On even-numbered days, all animals were given two 30min presentations of water separated by a 15min latency. The EU designated groups were given an injection of LiCl (81mg/kg) and DCS or SAL (refer to Table 2). The CSO designated groups were given an injection of SAL and DCS or SAL (refer to Table 2).

SAC-exposure Latency and Spontaneous Recovery Test

- After an animal reached the asymptotic EXT criterion, it was given two 30min presentations of water only (separated by 15min) every day for 29 days. No injections were given during this time.
- Thirty days following EXT, animals were presented with 0.3% SAC for 30min as a test of SR.

Results

Experiment 1: *Does DCS change the ability of rats to taste Saccharin or feel LiCl-induced malaise?*

Figure 1: *The doses of DCS tested did not disrupt the ability of rats to discriminate between 2 concentrations of saccharin*

* = Significant difference between volume of 0.3% and 0.6% Saccharin consumed

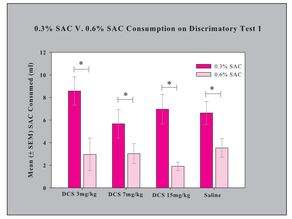
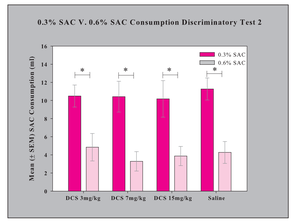
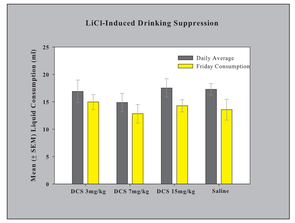


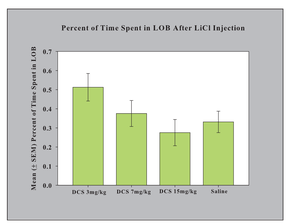
Figure 2: *Saccharin discrimination persisted during a second test and volumes of consumption rose from Test 1 to Test 2 – indicating that DCS did not act as a US and form a taste aversion.*

* = Significant difference between volume of 0.3% and 0.6% Saccharin consumed



Figures 3 & 4: *A 15 mg/kg (i.p.) dose of DCS did not attenuate LiCl-induced suppression of drinking nor did it affect the LiCl-induced "Lying-on-Belly" (LOB) measure.*





Experiment 2: Does DCS facilitate extinction of a CTA or eliminate SR?

Figure 5: *All animals formed a strong taste aversion following 3, SAC + LiCl pairings*

* = Significant decrease in saccharin consumed as compared to conditioning day 1

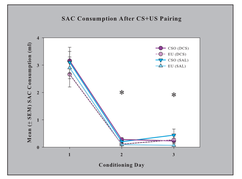


Figure 6: The EU extinction procedure significantly shortened the time to reach asymptotic extinction but DCS did not significantly alter this rate.

* = Significantly less than CSO extinction groups

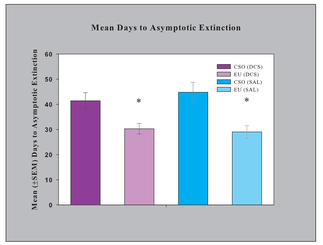


Figure 7: Most of the variance between times spent in extinction occurred during the static (initial) phase. Independent of DCS treatment, rats that underwent EU extinction reduced their time in the static phase.

* = Significantly less than SAL (CSO) extinction group

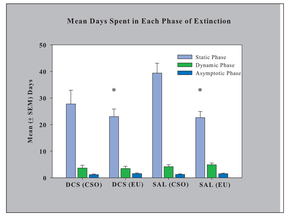
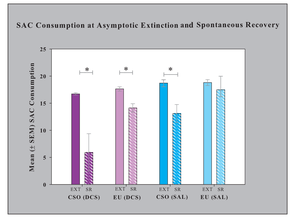


Figure 8: *Consistent with previous work (Mickley et al., 2009), rats that underwent the CS-only extinction procedure exhibited a significant spontaneous recovery of their CTA. Saline-treated rats that underwent EU extinction did not. However, DCS treatments of rats during EU extinction produced a significant SR in these animals.*

* = Groups showing spontaneous recovery of the CTA, i.e., Significantly less SAC consumed at SR test as compared to asymptotic extinction.



Summary & Conclusions

- As reported previously (Mickley et al., 2009), the EU extinction procedure significantly reduced the time for rats to achieve asymptotic extinction of a CTA and also attenuated SR of the aversion.
- However, DCS failed to shorten the time required to reach asymptotic EXT when either method (CS-only or EU) was employed.
- Both CSO and EU rats that received DCS treatments exhibited a SR of the CTA, suggesting that the drug not only failed to facilitate EXT but may also enhance the SR of this defensive reaction to a learned fear.
- Additional control experiments confirmed that an acute injection of 15 mg/kg (i.p.) DCS does not eliminate the rat's ability to discriminate between 0.3% and 0.6% SAC, it does not act as an effective US, nor does it alter the malaise inducing potency of LiCl. Thus, the drug effects we report here are unlikely the result of changes in our animals' ability to taste or DCS's potentiation of LiCl-induced malaise.
- These data raise doubts about the general efficacy of chronic DCS treatments as a means to facilitate extinction of defensive reactions to conditioned fears.
- The data are consistent with others (Parnas et al. 2005) indicating that multiple exposures to DCS can reduce its effectiveness as a facilitator of extinction learning.

References

Basoglu, M., Salcioglu, E. & Livanou, M. (2007). A randomized controlled study of single-session behavioral treatment of earthquake-related post-traumatic stress disorder using and earthquake simulator. *Psychological Medicine*, 37, 203-213.

Bouton, M.E. (2002). Context, ambiguity, and unlearning. Sources of relapse after behavioral extinction. *Biological Psychiatry*, 52, 976-986.

Davenport, R.A. and Houpt, T.A. (2009) D-cycloserine enhances short-delay, but not long-delay, conditioned taste aversion. *Pharmacology, Biochemistry and Behavior*, 91, 596-603.

Foa, E.B. (2000). Psychosocial treatment of post-traumatic stress disorder. *Journal of Clinical Psychiatry*, 61, 49-51.

Ledgerwood, L., Richardson, R. & Cranney, J. (2003). Effects of D-cycloserine on extinction of conditioned freezing. *Behavioral Neuroscience*, 117, 341-349.

Ledgerwood, L., Richardson, R. & Cranney, J. (2004). D-cycloserine and the facilitation of extinction of conditioned fear: consequences for reinstatement. *Behavioral Neuroscience*, 118, 505-513.

Meachum C.L. & Bernstein I.L. (1990) Conditioned responses to a taste conditioned stimulus paired with lithium chloride administration. *Behavioral Neuroscience*, 104, 711-5.

Mickley, G.A., Kenmuir, C.L., McMullen, C. A., Yocom, A. M., Valentine, E. L., Dengler-Crish, C.M., Weber, B., Wellman, J. A. & Remmers-Roeber, D. R. (2004). Dynamic Processing of Taste Aversion Extinction in the Brain. *Brain Research*, 1061/1, 79-89.

Mickley, G.A., Kenmuir, C.L., Yocom, A.M., Wellman, J.A., & Biada, J.M. (2005). A Role for prefrontal cortex in extinction of a conditioned taste aversion. *Brain Research*, 1051, 176-182.

Mickley, G.A., Hoxha, Z., Baeik, S., Kenmuir, C.L., Wellman, J.A., Biada, J.M. & DiSorbo, A. (2007). Spontaneous recovery of a conditioned taste aversion differentially alters extinction-induced changes in c-Fos protein expression in rat amygdala and neocortex. *Brain Research*, 1152, 139-157.

Mickley, G.A., Disorbo, A., Wilson, G.N., Huffman, J., Bacik, S., Hoxha, Z., Biada, J.M. and Kim, Y.-H. (2009). Explicit disassociation of a conditioned stimulus and unconditioned stimulus during extinction training reduces both time to asymptotic extinction and spontaneous recovery of a conditioned taste aversion. *Learning & Motivation*, 40, 209-220.

Nunnink, M., Davenport, R.A., Orega, B. & Houpt, T.A. (2007). D-cycloserine enhances conditioned taste aversion learning in rats. Pharmacology, *Biochemistry and Behavior*, 87, 321-330.

Nolan, L.J., McCaughey, S.A., Giza, B.K., Rhinehart-Doty, J.A., Smith, J.C., & Thomas, R.S. (1997). Extinction of a conditioned taste aversion in rats: I. Behavioral effects. *Physiology and Behavior*, 61, 319-323.

Parnas, A.S., Weber, M. and Richardson, R. (2005) Effets of multiple exposures to D-cycloserine on extinction of conditioned fear in rats. *Neurobiology of Learning & Memory*, 83, 224-231.

Thomas, B.L., Longo, C.L. & Ayres, J.J.B. (2005). Thwarting the renewal (relapse) of conditioned fear with the explicitly unpaired procedure: Possible interpretations and implications for treating human fears and phobias. *Learning and Motivation*, 36, 374-407.

Walker, D.L., Ressler, K.J., Lu, K.T. & Davis, M. (2002). Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine assessed with fear potentiated startle in rats. *The Journal of Neuroscience*, 22, 2343-2351.

Acknowledgements

Supported by NIMH grant: 2-R15-MH063720-03.

The authors would like to acknowledge the following students and technicians for their contributions to this work: Sarah Clark, Jennifer Danger, Sarah Frischmann, Jennifer Graebert, Nick Grisak, Natalie Hogan, Kyle Ketchesin, Bruce Kinley, & Dave Revta.