Acute, But Not Chronic, Exposure to D-Cycloserine Facilitates Extinction of a Conditioned Taste Aversion

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Abstract

D-cycloserine (DCS), the glutamate NMDA receptor partial agonist, has been reported to facilitate the extinction of learned fears. However, chronic exposure to the drug throughout the extinction (EXT) process does not facilitate the attenuation of a conditioned taste aversion (CTA) (Mickley et al., 2009). In the current study we evaluated the ability of acute treatments with DCS, given during different stages in the EXT process, to modulate the disappearance of behavioral indicators of a CTA.

Twenty-three hour water-deprived male Sprague-Dawley rats acquired a strong CTA following 3 CS-US pairings [0.3% saccharin (SAC) and 81 mg/kg (i.p.) Lithium Chloride (LiCl)]. 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. Previous studies have indicated that Spontaneous Recovery (SR) of a CTA emerges following CSO EXT but the EU-EXT paradigm causes a suppression of SR (Mickley et al., 2009). In the acute drug manipulation, DCS (15 mg/kg, i.p.) or saline control injections were administered, for 4 days only, immediately after daily liquid presentations (SAC or water, alternate days). This was done during one of 3 different phases of EXT training (i.e., 2-5%, 8-16% or 20-40% SAC reacceptance). Other animals, assigned to the chronic DCS condition, received daily DCS (15 mg/kg, i.p.) throughout EXT. Changes in SAC drinking in these animals were compared to the data from rats that received no drug (saline controls).

In a replication of our previous work, rats that went through the EU-EXT procedure achieved asymptotic extinction (81% of baseline SAC drinking) more quickly than did the CSO rats. However, chronic DCS exposure did not decrease the time required to reach asymptotic EXT when either method was employed. Conversely, CSO and EU-EXT rats having acute DCS treatments, extinguished their CTA more rapidly than did those animals exposed to DCS throughout EXT. CSO rats in the acute DCS treatment groups also reliably extinguished their CTA more quickly than did saline-control animals. Both CSO and EU rats that received chronic DCS treatments exhibited a SR of the CTA - suggesting that long-term treatment with the drug not only fails to facilitate EXT but may also enhance the SR of this defensive reaction to a learned fear. Acute DCS treatments were more effective in reducing SR than were chronic drug treatments and the timing of these acute DCS treatments affected SR of the CTA. Acute DCS administrations later in EXT were more effective in reducing SR than were early administrations. Additional control experiments confirmed that an acute injection of 15 mg/kg (i.p.) was not an effective US and did not change the animal's ability to taste SAC. Therefore, the drug effects we report here are unlikely the result of DCS-induced changes in the sensorium of our subjects.

These data agree with other findings (Vengeliene et al., 2008; Norberg et al., 2008) suggesting that DCS treatments are more effective when administered a limited number of times within a simple CSO extinction paradigm. Our data extend these findings and further suggest that acute exposure to DCS can also reduce SR of a CTA and speed up EU-EXT. While the timing of the acute DCS treatment during EXT is generally less important than its duration, it's important to note that the timing of acute DCS treatments during EXT can affect SR of a CTA.

Introduction

Fears may be acquired through associations of previously neutral stimuli with painful or aversive experiences - yielding phobias or post-traumatic stress disorder (Bouton, 2002; Thomas, Longo & Ayres, 2005).

Fears may be reduced (extinguished) 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).

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 avoidance of the CS 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 (EU) 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).

D-Cycloserine (DCS), a partial NMDA agonist at the strychnine-insensitive glycine site, has been shown to enhance CTA conditioning (Nunnink, Davenport, Ortega & Houpt, 2007; Davenport & Houpt, 2009) and extinction in CTA (Yu et al., 2009; Akirav et al., 2009) as well as other fear-based behavioral models (Ledgerwood, Richardson & Cranney, 2003 & 2004; Walker et al., 2002). However, the administration parameters governing DCS's effectiveness have not been well characterized.

We have recently shown that chronic exposure to DCS throughout the extinction (EXT) process does not facilitate the extinction of a conditioned taste aversion (CTA) (Remus et al., In Review).

Furthermore, it has been shown that 5 or more DCS exposures can cause a reduction in the memory-enhancing effects of this drug (Parnas, Weber & Richardson, 2005).

Therefore, the current study investigated the efficacy of acute DCS administration (4 exposures) 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 the CS and US were explicitly unpaired* ***(EU-EXT*** *procedure).*

Methods

Experiment 1: *Effects of chronic DCS administration on CTA extinction*

Table 1: Experiment 1 Group Nomenclature and Treatments

Group Nomenclature	N at EXT/SR	Conditioning		Extinction	
		Days 1, 3, 5	Days 2, 4, 6	Odd Days	Even Days
CSO(SAL)	10/5	SAC[1] + LiCl[2]	Water	SAC + SAL[3]	Water + (SAL + SAL)[4]
CSO(DCS)	11/5	SAC + LiCl	Water	SAC + DCS	Water + (SAL + DCS[5])
EU(SAL)	9/6	SAC + LiCl	Water	SAC + SAL	Water + (LiCl + SAL)
EU(DCS)	10/7	SAC + LiCl	Water	SAC + DCS	Water + (LiCl + DCS)

[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 (1ml/kg, i.p) which were 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)

CTA Acquisition

- Naïve adult 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 30-min access to 0.3% SAC and immediately injected with LiCl (81mg/kg, i.p.).
  - 15-min following SAC exposure animals were given 30-min access to water.
  - Days 2, 4, and 6 served as rest days on which the animals were given water only and no injections.

CTA Extinction

- Animals were given a 30-min presentation of 0.3% SAC, followed 15min later by 30-min access to water, every-other day (odd days; refer to Table 1) throughout EXT until they reached 81% 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 30-min presentations of water separated by a 15-min latency. The **EU** designated groups were given an injection of LiCl (81mg/kg) and DCS or SAL (refer to Table 1). The **CSO** designated groups were given an injection of SAL and DCS or SAL (refer to Table 1).

Experiment 2: *Effects of acute DCS administration on extinction*

Table 2: Experiment 2 Group Nomenclature and Treatments

Group Nomenclature	N at EXT/SR	Conditioning		Extinction	
		Days 1, 3, 5	Days 2, 4, 6	Odd Days	Even Days
CSO(2-5%)	9/4	SAC[1]+ LiCl[2]	Water	SAC + DCS	Water + (SAL +DCS)[4]
CSO(8-16%)	6/2	SAC + LiCl	Water	SAC + DCS	Water + (SAL +DCS[5])
CSO(20-40%)	5/4	SAC + LiCl	Water	SAC + DCS	Water + (SAL + DCS)
EU(2-5%)	10/5	SAC + LiCl	Water	SAC + DCS	Water + (LiCl + DCS)
EU(8-16%)	8/4	SAC + LiCl	Water	SAC+DCS	Water + (LiCl+ DCS)
EU(20-40%)	7/5	SAC + LiCl	Water	SAC+DCS	Water + (LiCl + DCS)

[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 (1ml/kg, i.p) which were 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)

CTA Acquisition

- Same as chronic DCS study

Extinction

- Animals were given 0.3% SAC for 30-min, followed by a rest period/injection time of 15 minutes. They were then given 30-min access to water. This occurred every-other day (odd days; refer to Table 2) throughout EXT until they reached 81% of baseline SAC consumption (designated "asymptotic" extinction; See Nolan et al., 1997).
- On odd-numbered days animals received an injection of DCS (15mg/kg) for four days once they reached their assigned SAC consumption range.
- On even-numbered days, all animals were given two 30-min presentations of water separated by a 15-min latency. The **EU** designated groups were given an injection of LiCl (81mg/kg) and DCS (once consumption range was reached) or SAL (refer to Table 2). The **CSO** designated groups were given an injection of SAL and DCS or SAL (refer to Table 2).

Results

Three SAC + LiCl pairings produced a significant Conditioned Taste Aversion (CTA; i.e., a decline in SAC drinking) in all animals

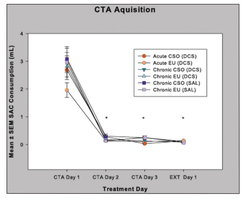

Figure 1: Animals in all treatment groups formed a strong taste aversion following three SAC+LiCl pairings.

* = Significant decrease in saccharin consumed as compared to conditioning day 1 ($p \leq 0.05$).

CTA extinction curves usually take on a sigmoid shape with the most time spent in the static phase when very little or no SAC consumption occurs

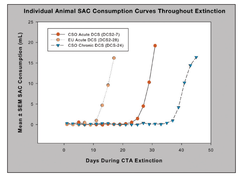

Figure 2: Individual extinction curves representative of the CSO Acute DCS, EU Acute DCS, and CSO Chronic DCS groups. Animals in the EU Acute DCS group extinguished faster than animals in the CSO Acute DCS group. Animals in the CSO Acute DCS group extinguished faster than animals in the CSO Chronic DCS group.

Explicit unpairing of the CS and US shortens the extinction of a CTA. Chronic DCS exposure does not affect the pace of extinction.

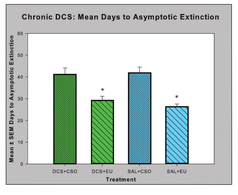

Figure 3: The EU extinction procedure significantly shortened the time it took animals to reach asymptotic extinction of a CTA. But chronic exposure to DCS throughout the EXT process did not significantly alter this rate. * = Significantly less than CSO extinction groups ($p \leq 0.05$).

The timing of acute DCS exposures does not affect CTA extinction time

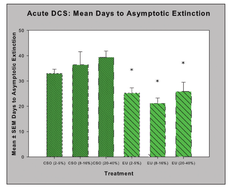

Figure 4: The EU procedure reduced the time to CTA extinction in rats that received acute 4-day exposure to DCS. However, the timing of DCS treatment did not affect the CTA extinction process. * = Significantly less than CSO extinction groups ($p \leq 0.05$).

Acute DCS treatments, but NOT chronic DCS treatment, significantly shortened CTA extinction

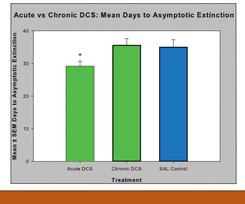

Figure 5: Overall, acute exposure to DCS significantly decreased the time to reach asymptotic extinction compared to both chronic DCS exposure, as well as saline control animals. * = Significantly less than the Chronic DCS group and SAL Control group ($p \leq 0.05$).

Acute DCS administration is the most effective in facilitating CTA extinction when given in the context of a CSO extinction paradigm

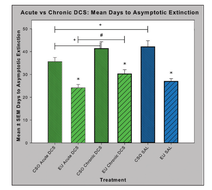

Figure 6: An acute exposure of DCS to animals in the CSO extinction paradigm shortened the time to asymptotic extinction compared to both the chronically treated DCS CSO animals and the saline control CSO animals (+ $\leq$ 0.05).

An acute exposure of DCS in EU animals shortened the time to asymptotic extinction compared to chronically treated DCS EU animals (# $\leq$ 0.05) but not EU saline-treated control animals.

Overall, the EU procedure significantly decreased days to asymptotic extinction for the DCS acute, DCS chronic, and saline control animals. * = Significant decrease ($p \leq 0.05$) compared to adjacent CSO treatment group.

Timing of the acute DCS treatments affected SR of the CTA. Acute DCS administrations later in EXT were more effective in reducing SR than were early administrations.

Figure 7: SR of the CTA occurred in rats that received acute DCS treatments early during CSO (2-5% & 8-16%) or EU (2-5%) EXT training. However, administration of DCS later in EXT eliminated SR of the CTA. * = Significant SR of a CTA, i.e., SAC consumed at SR test is significantly less than SAC consumed at asymptotic EXT ($p \leq 0.05$).

Acute DCS reduces SR of an extinguished CTA. SR is prominent in EU extinguished rats given chronic DCS.

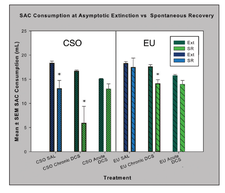

Figure 8: Consistent with previous work (Mickley et al., 2009), rats that underwent the CS-only extinction procedure exhibited a significant spontaneous recovery of their CTA. Saline-treated rats that underwent EU extinction did not. However, chronic DCS treatments of rats during EU extinction produced a significant SR in these animals. Acute DCS reduces SR of a CTA in CSO rats. * = Significant SR of a CTA, i.e., SAC consumed at SR test is significantly less than SAC consumed at asymptotic EXT ($p \leq 0.05$).

Suppression SAC consumption at SR test: Rats treated with chronic DCS during extinction exhibited a SR of a CTA that is comparable to saline-treated rats and more prominent than rats treated acutely with DCS.

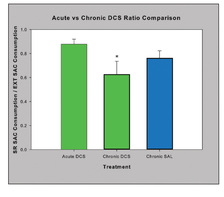

Figure 9: The mean ($\pm$ SEM) ratio of "SAC consumed at SR test" / "SAC consumed at asymptotic extinction" provides an indicator of SR potency (1 = No SR). Rats treated with chronic DCS during extinction (N=12) exhibited a SR that is comparable to saline-treated rats (N=9) and more prominent than rats treated acutely with DCS (N=22). * = Significantly lower suppression ratio ($p \leq 0.05$) than rats acutely treated with DCS.

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.

However, chronic DCS failed to shorten the time required to reach asymptotic EXT when either method (CS-only or EU) was employed.

Acute DCS treatments, but not chronic DCS treatment, shortened the time to reach asymptotic extinction. Moreover, acute DCS treatments were more effective in reducing SR of a CTA than were chronic drug treatments.

The timing of the acute DCS treatments during extinction did not affect the days required to achieve asymptotic EXT but it did affect SR of the CTA.

Acute DCS administrations later in EXT were more effective in reducing SR than were DCS doses given early in the EXT process.

Acute DCS administration given later in EXT training appears to be most effective in facilitating CTA extinction and reducing SR.

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.

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