

**D-cycloserine Facilitates Extinction of Cocaine Self Administration in Rats**

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**Abstract**

Previous research has indicated that d-cycloserine (DCS; a NMDA partial agonist) facilitates the extinction of conditioned fear as well as the extinction of cocaine conditioned place preference (CPP). Sprague Dawley rats were first trained to self-administer (SA) cocaine and then we compared their extinction behavior (lever pressing) following treatment with vehicle; 15mg/kg DCS; or 30 mg/kg DCS. We showed that 30 mg/kg DCS, but not 15 mg/kg significantly accelerated extinction of cocaine self-administration behavior when compared with saline by almost half (4 days versus 9 days). At 2 weeks when all animals had extinguished there were no longer differences between the groups. The present findings support of the potential of NMDA partial agonists as prospectively valuable in facilitating the extinction of cocaine seeking behavior. More specifically we demonstrate that 30mg/kg DCS was effective at significantly accelerating the extinction of cocaine self-administration behavior in rats. These results provide further support for the potential of DCS as a treatment strategy for addiction.

## 1. Introduction

It is estimated that over 6 million individual in the United States, ages 12 and older have been exposed to cocaine (SAMHSA, 2007). Cocaine increases dopamine in the nucleus accumbens by blocking dopamine transporters, which is considered the mechanism by which it induces rewarding effects that can result in addiction (Ritz et al. 1987). Dopamine is involved with reward and prediction of reward and with conditioned learning. Dopamine modulation of the amygdala and the prefrontal cortex predominantly through D1 receptors are implicated in conditioning (Beninger and Gerdjikov, 2004) through neuroplastic adaptations in glutamatergic neurotransmission (Crombag et al., 2002; Kalivas and Volkow, 2005).

Specifically changes in expression of AMPA (alpha-amino-3-hydroxy-5-methylisoxazole propionic acid) and NMDA (N-methyl-D-aspartate) receptors, which contribute to the neuroplastic processes linked with learning and memory including long-term potentiation are involved in conditioning (Rao and Finkbeiner, 2007). Both NMDA and AMPA receptors are implicated in cocaine-seeking behavior controlled by drug-associated cues (Di Ciano and Everitt, 2001). Acute and chronic cocaine potentiate synaptic strength in the ventral tegmental area (VTA) through changes in AMPA receptors, which is blocked by NMDA receptor antagonists (Borgland et al., 2004).

The involvement of NMDA and AMPA receptors in cocaine addiction has raised interest in the therapeutic potential of medications that target NMDA and/or AMPA receptors. D-cycloserine, (DCS), a partial NMDA receptor agonist (Klodzinska and Chojnacka-Wojcik, 2000) has been shown to facilitate extinction of previously conditioned fear and anxiety, both in pre-clinical and clinical models (Davis et al., 2006; Guastella et al.,

2007; Parnas, 2005; Ressler et al., 2004) (Faraone et al., 2005; Ressler et al., 2004; Vervliet, 2008); furthermore, recent findings showed that DCS facilitated extinction of cocaine conditioned place preference (CPP) in rats (Botreau et al., 2006). The facilitated extinction was interpreted to reflect DCS's enhancement of memory consolidation during the extinction conditions, via its effects on NMDA receptors (Botreau et al., 2006; Vervliet, 2008).

We recently showed a similar effect of DCS in extinction to CPP for cocaine in mice that was dose related and persisted for up to 2 weeks (Thanos et al., 2009). Specifically, extinction to cocaine CPP was significantly faster with DCS than with vehicle treatment (3 sessions, versus 6 sessions respectively). After extinction was achieved; mice were retested for CPP 1 and 2 weeks later. All animals maintained extinction to CPP one week later but at 2 weeks whereas the vehicle and the 15mg/kg DCS treated animals maintained the extinction the 30mg/kg DCS treated mice had renewed CPP and also showed inhibited locomotor activity. Though these results corroborated in mice the previously reported acceleration of extinction to cocaine induced CPP by DCS in rats we also show that the higher DCS doses had the opposite effect; that is it facilitated CPP reestablishment after extinction. Thus while DCS could be beneficial in accelerating the extinction to conditioned responses in addiction, at higher doses, it could increase the risk of relapse. This highlights the importance of evaluating not only the short term beneficial therapeutic effects of DCS but also of evaluating the potential of longer lasting undesirable effects.

The purpose of this study was to further investigate the possible psychotherapeutic applications of DCS on the extinction of a rat model of cocaine self-

administration. The self-administration model provides qualitative and quantitative measure of voluntary cocaine consumption analogous to the clinical situation and provides insight into a different aspect of drug abuse and addiction (motivational drive to exert the behavior) (Dykstra et al., 1997; Schuster and Johanson, 1988; Solinas et al., 2006) that distinct from CPP (conditioned behavior).

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## **2. Materials and Methods**

### **2.1. Animals**

Thirty six, male 6-week, old Sprague–Dawley rats (Taconic Farms) were allowed seven days to habituate to experimental conditions: temperature ( $72\pm 2^{\circ}\text{F}$ ); controlled humidity (40-60%); a twelve hour reverse light cycle (lights off at 07:00 hours). Rats were individually housed and kept on an ad lib diet. For the cocaine self-administration experiments, only rat chow was restricted (18 g/day) to maintain a stable body weight, which was recorded daily throughout the experiment. All experiments were conducted in an accredited animal husbandry facility that was approved by the Association for Assessment of Laboratory Animal Care, and the Institutional Animal Care and Use Committee of Brookhaven National Laboratory.

### **2.2. Drugs**

Cocaine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was prepared by dissolving it in 0.9% saline for doses of 0.750 mg/kg and 0.375 mg/kg for intravenous (i.v.) infusion. All cocaine solutions were infused i.v. at a volume of 100  $\mu\text{l}$ . D-cycloserine (Sigma-Aldrich, St. Louis, MO, USA) was used in a 15mg/kg dose (1.5 M; 10 ml/kg i.p.) and a 30mg/kg dose (3 M; 10 ml/kg i.p.) during extinction. Saline (0.9% NaCl) was used (10 ml/kg i.p) as the vehicle solution. Rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) given i.p for surgery under aseptic conditions

### **2.3. Apparatus**

The self administration apparatus (Habitest - Coulbourn Instruments; Allentown, PA) was placed inside a rigid foam sound attenuated cubicle equipped with a 28 volt exhaust fan. Each operant chamber contained a horizontal grid floor with metal side walls and clear front and back walls. One side wall contained two levers and a food receptacle in the center. The left lever was designated as the active lever while the right lever was the inactive lever. Both levers were situated directly under their respective cue lights. The back wall was equipped with an infrared activity monitor that collected locomotor behavior. Attached to a swivel arm, the infusion line entered the chamber from the center of the ceiling to be connected to the catheter on the rat for drug delivery. The cocaine was injected i.v. through the infusion line with an infusion pump at a fixed rate of 0.025 ml/s for duration of 4 s. All experimental variables were programmed and controlled using Graphic State Version 3.02 software that allowed for behavioral data collection.

### **2.4. Tests and Procedures**

#### **2.4.1. Food Training**

Rats were trained to respond to an operant lever response task for a food pellet prior to catheterization. Training sessions were conducted in the dark cycle from 8:00h to 15:00h and lasted for 4 days in 90min daily sessions. A fixed-ratio 1 (FR1) reinforcement schedule with a 30s timeout period was used. Pressing the active lever once released one (45mg) food pellet into the food receptacle as the cue light was illuminated for a 30s timeout period. During the timeout period, food was not released but the response recorded. Pressing the inactive lever had no programmed consequence.

Successful lever discrimination was achieved when rats met previously described criterion of an active/inactive lever press ratio  $\geq 2:1$  (Larson and Carroll, 2005). When rats exhibited lever discrimination they underwent surgery for catheterization. After surgery and recovery, rats went through one additional session of food retraining to ensure conditioning met aforementioned criterion to be able to move onto the cocaine self-administration phase of the experiment.

#### *2.4.2. Jugular Vein Catheterization*

Indwelling catheters were implanted in the right jugular vein of the rats for i.v. cocaine self-administration. Rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) given i.p for surgery under aseptic conditions. The silastic tubing end of the catheter was introduced into the right jugular vein and secured with sutures. On the other end of the catheter, a 22G cannula guide (Plastics One, Roanoke, VA, USA) was routed subcutaneously along the right upper dorsum to the midscapular region. Following surgery, rats were given a 3 day recovery period. On every day after the surgery, the rats were given an i.v. injection through the catheter of cefazolin and glycerol/heparin solution to prevent occlusion and/or infection. The catheter patency was also tested daily by administering a mixture of ketamine (5 mg/kg, i.v.) and midazolam (0.75 mg/kg, i.v.). The catheter was determined to be patent only if the rat lost righting reflex within 3s of the injection. If the catheter was not patent, the rat was immediately taken out of the study.

#### *2.4.3. Cocaine Self-Administration*

Cocaine self-administration sessions (90 min/day) lasted for 15 days. All drug sessions were conducted in the dark cycle from 8:00h to 15:00h. A FR1 schedule was used with a 30s timeout period. Immediately before and after the session, catheters were injected with saline to prevent occlusion. At the start of every session, rats received one priming infusion of cocaine. A single press of the active lever resulted in an immediate delivery of cocaine (0.75 mg/kg/infusion, i.v.) and a 30s timeout period. During the timeout period, the cue light above the active lever was illuminated and the drug was not available. Lever presses were recorded during the timeout period. Inactive lever pressing during the session did not have a programmed consequence, but presses were recorded. During the first 7 days of cocaine self-administration, rats received an i.v. dose of 0.75 mg/kg/infusion cocaine in a volume of 0.1ml with a FR1 schedule. For the last 8 days, the i.v. dose was halved to 0.375 mg/kg/infusion of cocaine under the FR1 schedule to look at the sensitivity in the dose response rate.

#### *2.4.4. Cocaine Extinction*

The extinction procedures mimicked the self-administration procedures. Rats were placed in the operant chambers for extinction sessions (90 min/day) for 15 days. All extinction sessions were conducted in the dark cycle from 8:00h to 15:00h. During the extinction session, a single press of the left lever (active lever) resulted in an immediate delivery of saline. All other parameters from the self-administration sessions remained the same.

### **2.5. Groups**

At the end of the self-administration phase, and prior to the start of the extinction phase; the rats were randomly assigned into three groups: vehicle (control), low dose DCS (15 mg/kg i.p. DCS) or high dose DCS (30 mg/kg i.p. DCS). Immediately after the end of each extinction session, the rats were injected with the vehicle solution, 15 mg/kg i.p. DCS, or 30 mg/kg i.p. DCS.

### **2.6. Statistics**

Two-way Analysis of Variance (ANOVA, followed by pair-wise comparisons using the Holm-Sidak method) was utilized in the analysis of the self-administration, extinction and locomotor activity data for both treatment and session as the variables. All statistical comparisons were performed using the SigmaStat 3.1 statistical software.

### 3. Results

#### 3.1.1. Food Training

Food training (figure 1) allowed for the rats to acquire the ability to differentiate between the active lever and the inactive lever. A two-way ANOVA revealed a significantly greater number of active versus inactive lever presses [ $F(1, 3) = 12.04$ ;  $p < 0.001$ ; figures 2 and 3]. Pair-wise multiple comparisons (Holm-Sidak method) indicated that this difference was significant beginning at day 3 ( $t = 3.28$ ;  $p < 0.001$ ; figure 2 and 3).

#### 3.1.2. Cocaine Self-Administration

Rats as expected showed significantly greater number of cocaine (active) lever presses compared to the inactive lever which was maintained throughout the 15 days [2-way ANOVA;  $F(1, 23) = 300.53$ ;  $p < 0.001$ ; figures 2 and 3]. During the first week (days 5-11; figure 1), rats lever pressed for 0.75 mg/kg i.v. cocaine/ infusion (compared to the inactive lever). Similarly during the second week, (days 12-19), rats again preferred and showed more lever presses for 0.35 mg/kg i.v. cocaine/ infusion (figure 2). A 2-way ANOVA showed a significantly greater number of [ $F(1, 23) = 31.34$ ;  $p < 0.001$ ; figure 2] lever presses on days 12-19 (at 0.0375 mg/kg/infusion) as compared to the previous week (at 0.75 mg/kg/infusion). Furthermore, a 2-way ANOVA showed as expected a significantly greater [ $F(1, 23) = 300.53$ ;  $p < 0.001$ ] number of active lever presses during cocaine self administration compared to the inactive lever presses.

Pair-wise multiple comparisons indicated that there was a significantly greater number of active lever presses at both doses ( $t = 9.10$ ;  $p < 0.001$ ; 0.75 mg/kg cocaine during days 5-11 and at 0.375mg/kg cocaine during days 12-19;  $t = 15.53$ ;  $p < 0.001$  figures 1-3).

### 3.1.3. Extinction: Active Lever

A 2-way ANOVA used to examine active lever pressing during the extinction period (figure 1; days 20-34) yielded a significant difference in the number of active lever presses between the 3 treatment (low and high dose DCS, and vehicle) groups [ $F(1, 23) = 7.56$ ;  $p < 0.001$ ; figures 2 and 3]; as well as a significant difference between the number of days needed to achieve extinction [ $F(1, 23) = 3.89$ ;  $p < 0.001$ ; figure 2].

Pair-wise multiple comparisons (Holm-Sidak method) confirmed significant differences between the 15 mg/kg and 30 mg/kg i.p. DCS treatments ( $t = 2.85$ ;  $p < 0.01$ ), and between the vehicle and 30 mg/kg i.p. DCS treatments ( $t = 3.56$ ;  $p < 0.001$ ) favoring the higher DCS dose treatment of 30 mg/kg (figures 2 and 3). However, no statistical difference was obtained from the comparison between the vehicle and the 15 mg/kg i.p. DCS treatments ( $t = 0.65$ ;  $p > 0.5$ ). Furthermore, pairwise comparisons revealed significant differences ( $p < 0.05$ ; figure 2) in the 30mg/kg treated rats and vehicle on extinction days 21 – 26 [day 21 ( $t=6.04$ ); day 22 ( $t=3.16$ ); day 23 ( $t=4.02$ ); day 24 ( $t=3.12$ ); day 25 ( $t=2.01$ ); day 26 ( $t=2.16$ )]. Thus, 30 mg/kg i.p. DCS treated rats showed a significantly faster rate of extinguishing active lever responses.

### 3.1.4 Extinction: Inactive Lever

During the extinction period a 2-way ANOVA was used to examine inactive lever pressing (figure 1; days 20-34). The results indicated a statistical significance in the number of inactive lever presses between all 3 treatment (low and high dose DCS, and

vehicle) groups [ $F(1, 23) = 5.54$ ;  $p < 0.01$ ]. However, there was no significant difference found in the comparison of data among the extinction days [ $F(1, 23) = 0.94$ ;  $p > 0.05$ ].

Pair-wise multiple comparisons (Holm-Sidak method) confirmed significant differences between the 15 mg/kg i.p. DCS and vehicle treatments ( $t = 2.84$ ;  $p < 0.01$ ), and between the 30 mg/kg i.p. DCS and vehicle treatments ( $t = 2.52$ ;  $p < 0.01$ ).

However, no statistical difference was obtained from the comparison between the 15 mg/kg i.p. and 30 mg/kg i.p. DCS treatments ( $t = 0.24$ ;  $p > 0.5$ ).

#### 3.1.5. Locomotor

As expected, there was a significant increase in locomotor activity during the self-administration phase of the experiment compared to food training [ $F(1, 23) = 2.81$ ;  $p < 0.01$ ; figures 1 and 4]. During the extinction phase, a 2-way ANOVA yielded no significant difference in locomotor activity among the three treatment groups [ $F(1, 23) = 0.74$ ;  $p > 0.05$ ; figure 4].

#### 4. Discussion

Previous studies have examined the behavioral and cognitive effects (Ho et al., 2005; Land and Riccio, 1999; Myers and Davis, 2002) of DCS and have reported that DCS may be involved in the facilitation of new memory formation that oppresses or overrides previously conditioned memories. In addition, it has been reported that as an NMDA partial agonist, DCS plays an important role in the consolidation of memories (Richardson, 2004), long-term potentiation or LTP (Yaka et al., 2007) and cocaine potentiated synaptic strength in the VTA (Borgland et al., 2004).

Evidence of the therapeutic effects of DCS, on extinction; have been widely described with respect to the extinction of conditioned fear and anxiety (Hofmann et al., 2006; Norberg et al., 2008; Richardson, 2004). Most recently DCS has been examined for its properties of extinction of drug seeking behavior after repeated drug exposures. The present findings, along with those of recent studies (Boutreau et al., 2006; Thanos et al., 2009; Paolone et al. 2009), further support the notion that DCS may have therapeutic value for the extinction of cocaine seeking and abuse. Progressing from the original study on DCS-facilitated cocaine CPP extinction in rats (Boutreau et al., 2006) and mice (Thanos et al., 2009); the present study, utilizing a self-administration paradigm and two doses (15mg/kg or 30 mg/kg i.p. DCS), revealed the dose effect and efficacy of DCS on the extinction of cocaine self-administration in rats.

Rats displayed similar cocaine self-administration behavior during the maintenance portion of the study as previously reported (Mark et al., 1999). When the cocaine dose was decreased in week 2 of self-administration, rats showed a

corresponding increase in active lever responding for cocaine. Extinction of cocaine self-administration behavior in the rats indicated a progressive, daily, decrease in active lever responses; and slight increases in inactive lever responses. There were no significant differences in extinction between the vehicle (control) and the 15 mg/kg DCS rats. These results challenged those previously found in the extinction of a cocaine CPP (Botreau et al., 2006); study in which 15mg/kg DCS were found effective in the facilitation of a cocaine CPP extinction. This is likely to reflect the differences in the neuronal processes that are involved in CPP, which does not require behavioral output other than choice of space versus those involved in drug self-administration, which require lever pressing in order to get the expected cocaine. This could be interpreted to indicate that whereas the lower DCS (15mg/kg) dose may be sufficient to inhibit the place conditioning association it is insufficient to inhibit the motivational drive to lever press for cocaine.

The results from this study also indicated that there were differences in the rate at which the extinction took place between the vehicle (control) and the 30 mg/kg DCS treated rats. This higher DCS dose (30 mg/kg), showed a sharper decline in active lever responding and produced this effect rapidly (within the first treatment sessions). In addition, this effect was maintained throughout the first week extinction period (days 21–26). This facilitated cocaine extinction observed in the rats treated with 30mg/kg DCS is consistent with findings for this dose of DCS on the extinction of fear and anxiety (Anthony and Nevins, 1993) and with findings of extinction on cocaine self administration (Nic Dhonnchadha et al. 2010).

However, analysis of the inactive lever presses for all three treatment groups; during the extinction session indicate a somewhat erratic behavior. While an increased

number of inactive lever presses during the first few days of extinction is expected due to the initial cocaine seeking response the rats show; this response typically lasts a few sessions. Indeed, the vehicle treated rats in this study within 2 extinction sessions showed a similar number of inactive lever responses in the extinction phase as in the cocaine self administration phase. However, the DCS treated rats did show a greater number of inactive lever responses compared to the vehicle treated rats overall during extinction; although no significant difference was found on a day to day analysis. This increase in inactive lever responses during extinction of cocaine self-administration following DCS treatment has not been previously examined and further research is needed into its significance and mechanism. Thus, while DCS seems to facilitate the extinction of lever responding for cocaine; probably by enhancing learning of new contextual relationships during extinction sessions; this new learning of contextual relationships needs to be further examined in terms of responses to other cues.

Finally, there was no significant effect of DCS on locomotor activity throughout the extinction phase. This was in part consistent with the locomotor activity during CPP observed with 15mg/kg DCS (Thanos et al. 2009). In addition, while previous data in CPP had shown inhibition of locomotor activity at 30mg/kg, this was not observed here during the self administration extinction paradigm. This may be due to the differences in the methods used (40 min per session, 8 sessions of DCS treatment in the CPP study (Thanos et al., 2009); 90 min per session, 15 sessions of DCS treatment in the present study. While rats treated with DCS compared to control rats did not show significant locomotor side effects further studies need to examine long-term effects following DCS treatment beyond 2 weeks.

## 5. Conclusion

This study demonstrated that DCS (30mg/kg) can facilitate the extinction of the cocaine self-administration behavior in rats. These results when combined with data (from our lab and others) of facilitating cocaine CPP further supports the hypothesis that DCS may be useful in the treatment of drug abuse and addiction. Further investigations are required to evaluate the effects of DCS, it's dosing, and long-term effects on the extinction of drug abuse behaviors as well reinstatement testing. Future research will examine the whether these effects of DCS are selective for drug-seeking behavior or they are generalized to natural reinforcers such as sucrose or fatty foods.

While DCS is approved in humans as an antibiotic for the treatment of tuberculosis, the clinical significance of our results in cocaine-dependent patients is limited since it remains unclear if DCS exhibits generalized extinction (ie to other appetitive cues).

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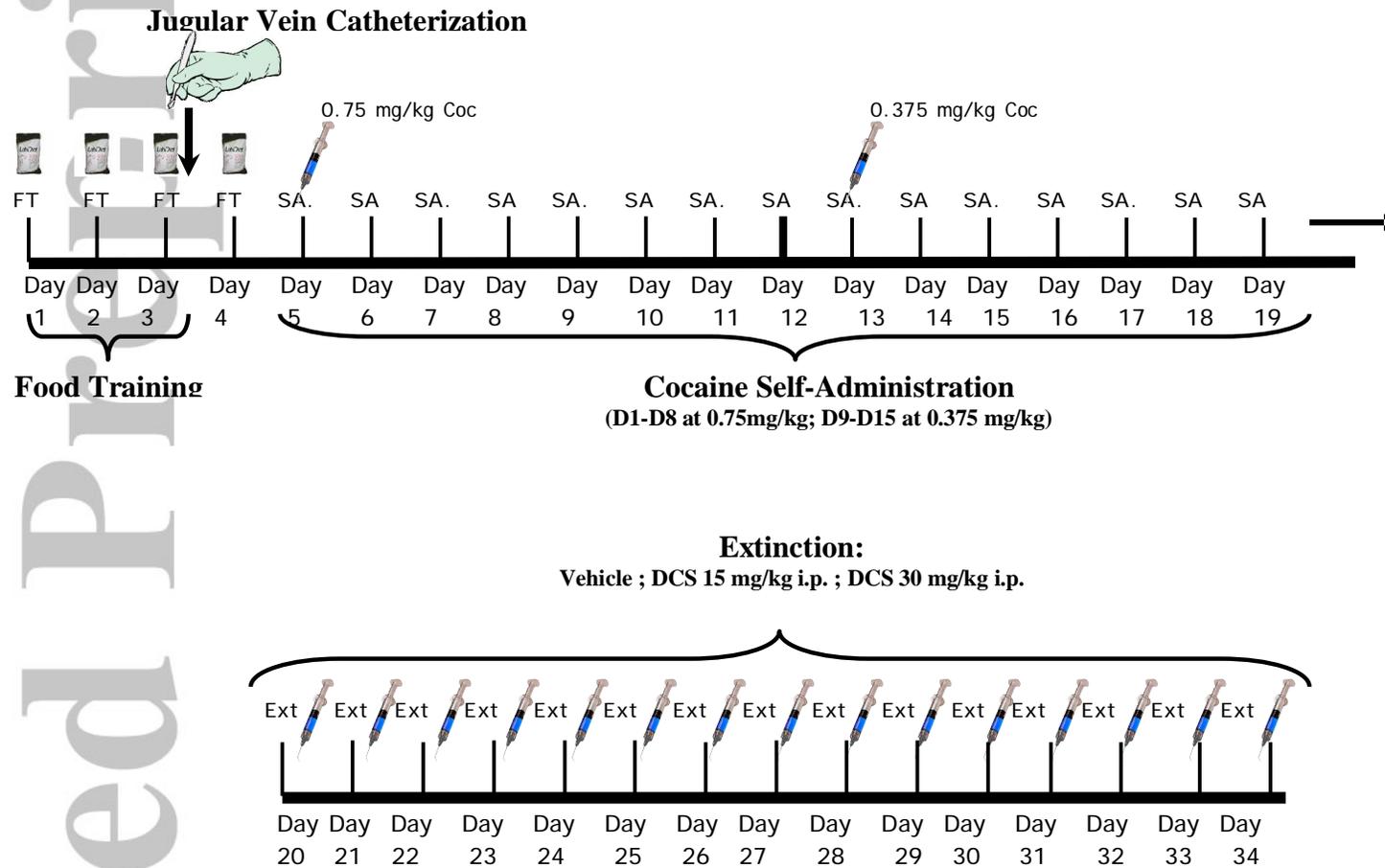
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## 7. Figure Legends

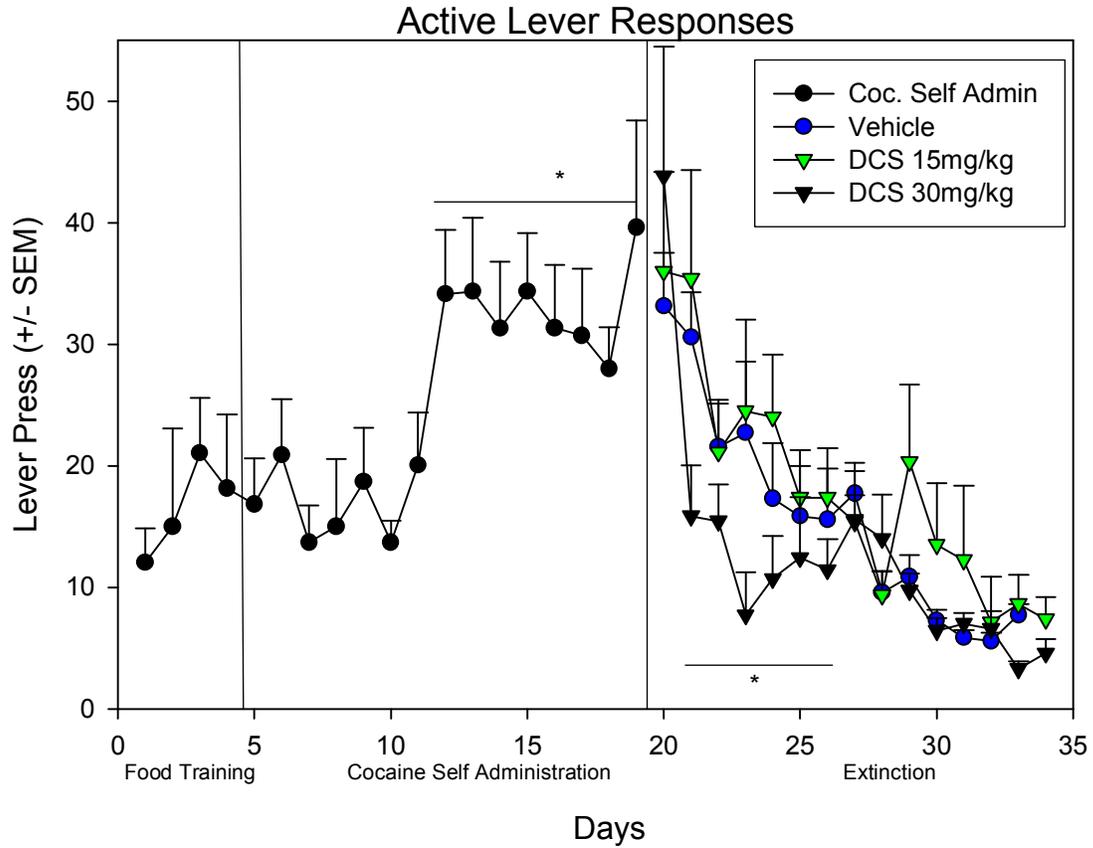
Figure 1. Study Timeline: Cocaine self-administration and extinction

- Figure 2. Active Lever Responses: Food Training, Cocaine Self-Administration, and Vehicle / 15 mg/kg i.p. DCS / 30 mg/kg i.p. DCS paired Extinction. (\*) indicates significant difference during the cocaine self-administrations sections; (\*\*) indicates significant difference for 30 mg/kg i.p. DCS treatment group.
- Figure 3. Inactive Responses:: Food Training, Cocaine Self-Administration, and Vehicle / 15 mg/kg i.p.DCS / 30 mg/kg i.p. DCS paired Extinction
- Figure 4. Locomotor activity during Food Training, Cocaine Self-Administration, and Vehicle / 15 mg/kg i.p.DCS / 30 mg/kg i.p. DCS paired Extinction: (\*) indicates significant difference in locomotor activity during the cocaine self-administration section.

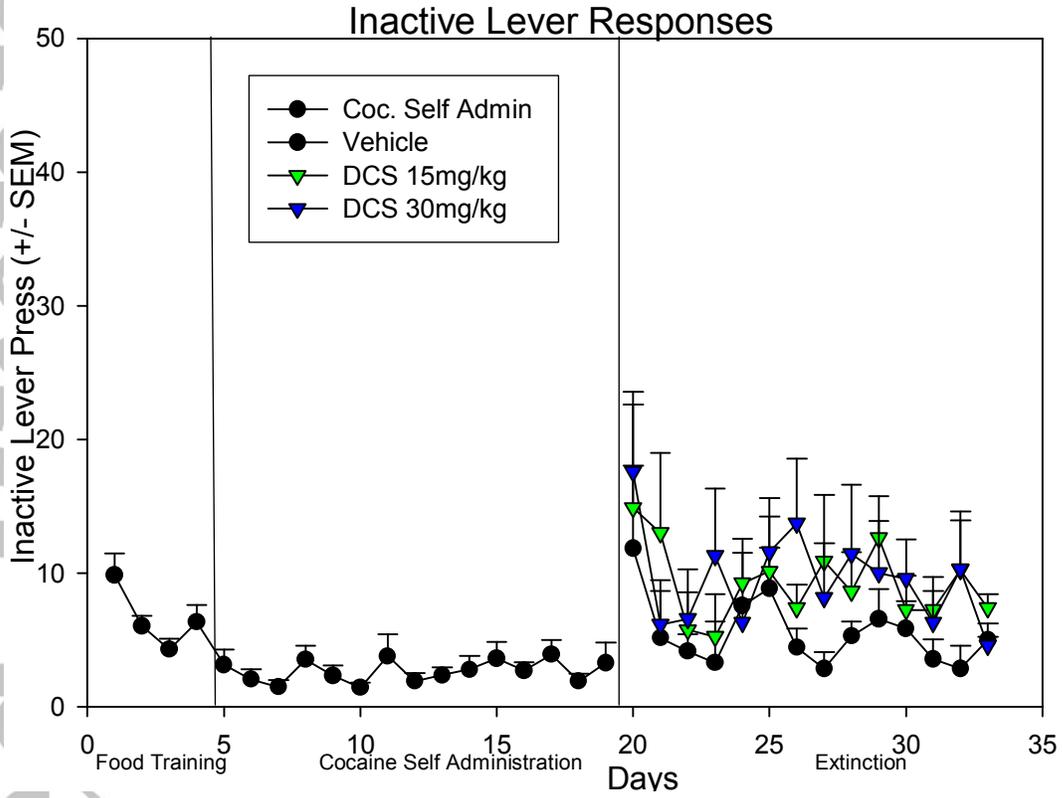
**Figure 1.** Timeline of Study: Cocaine self-administration and extinction.



**Figure 2** - Active Lever Responses: Food training, cocaine self administration and extinction



**Figure 3** - Inactive Lever Responses: Food training, cocaine self administration and extinction



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**Figure 4** – Locomotor Activity: Food training, cocaine self administration and extinction

