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## Low distress tolerance predicts heightened drug seeking and taking after extended abstinence from cocaine self-administration

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### Abstract

Distress tolerance (DT), defined as the ability to persist in goal-directed behavior while experiencing psychological distress, is associated with greater frequency of substance use and poor treatment outcomes. To examine a potential causal role substance use may play in DT, we developed a rodent model of DT in which rats had to press a lever within a continuously decreasing time window for reward, while receiving negative feedback on failure trials. DT was defined as the time rats continued to seek reward before quitting the task. We assessed the relationship of DT with cocaine seeking/taking by measuring DT before cocaine self-administration (SA), and after one week and one month of drug abstinence. We found that DT prior to cocaine SA did not predict cocaine seeking/taking, yet DT measured after one month abstinence significantly predicted subsequent high levels of early session cocaine taking. Additionally, high DT measured after abstinence protected against high cocaine seeking, but this protective effect was blocked in rats with high impulsivity. Finally, while a decrease in one month-abstinent DT was observed following SA across treatment conditions, among cocaine-exposed rats, greater cocaine self-administration correlated with a steeper decrease in DT. These results show that low DT after drug abstinence is associated with heightened levels of cocaine seeking and taking behavior, and that impulsivity influences this effect. Collectively, these results support the validity of our rodent DT model while extending the human literature, and set the foundation for future animal studies designed to determine neural mechanisms underlying DT.

### Keywords

Abstinence; Cocaine; Distress Tolerance; Impulsivity; Rat; Self-Administration

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## Introduction

Negative reinforcement models of addiction indicate that a motivational basis of drug use is the reduction or avoidance of physical and/or psychological distress (Baker et al., 2004; Koob, 2013). As a proxy for negative reinforcement behavior, laboratory paradigms assess distress tolerance (DT), defined behaviorally as the ability to persist in goal-directed behavior while experiencing psychological distress (Magidson et al., 2013). Behavioral measures indicate that low DT is associated with a greater frequency of substance use, and among treatment seeking substance users, greater likelihood of treatment dropout and relapse (Brown et al., 2002; Brandon et al., 2003; Daughters et al., 2005a,b; Brown et al., 2009; Strong et al., 2012).

In addition to negative reinforcement models of substance use, established literature emphasizes the role of motor impulsivity, defined broadly as an inability to inhibit behavioral responses, as a risk factor for substance use (Steinberg et al., 2008). Importantly, evidence indicates that one's ability to persist in goal-directed behavior while experiencing affective distress is influenced by top-down neural mechanisms (Daughters et al., 2016), which overlap with those implicated in impulsivity (Hu et al., 2015). As such, an examination of the unique and interactive role of DT and impulsivity on substance use is important to consider.

The ability of DT to predict subsequent relapse makes it a promising behavioral target, and a number of studies have developed DT-based treatment strategies (Brown et al., 2008; Bernalova et al., 2012). Although studies have begun to examine the neurobiology of DT (Daughters et al., 2016), the underlying mechanisms of DT remain poorly understood. Furthermore, the exact role of DT in addiction itself is not clear. For example, although studies on drug naive adolescents provide evidence suggesting a link between low DT and risk behavior, including alcohol use (Daughters et al., 2009; Cummings et al., 2013), it is unknown whether drug-naïve DT influences subsequent substance use, or, conversely, if substance use lowers DT. Indeed, long-term longitudinal studies are required to answer these questions within a human population.

Here, we took a translational approach to address these issues by developing a rodent model of DT. Our model is based on behavioral tasks used to measure psychological DT in humans, namely the Computerized Paced Auditory Serial Attention Task (PASAT-C, Lejeuz et al., 2003). Human participants engage in an increasingly difficult task, characterized by forced failure and negative feedback, with the belief that task performance is associated with a reward. Following a controlled amount of exposure to the task, the participant is then provided the option to continue engaging in the task to improve performance, or to quit, with DT measured as the duration of time the participants spend on this last level before quitting. In our model, we designed a reaction time procedure in which rats learn to respond within a brief time window to receive a reward. On test day, the time window narrows significantly, causing the animal to encounter repeated forced failure and negative feedback (white noise burst), similar to the human DT tasks. Our dependent measure is also the amount of time it takes each rat to quit responding in the task. Guided by the findings relating DT and motor impulsivity to substance use, we sought to determine the relationship between DT, motor

impulsivity and a model of substance use in rodents, namely 6-hour access to cocaine self-administration (Ahmed & Koob, 1998).

## Materials and Methods

### Subjects

Twenty-one male Long Evans rats (Charles River, Raleigh, NC) initially aged 8–10 weeks and weighing 300–325 g were used. Rats were individually housed with a 12h/12h light-dark cycle (lights on at 7 PM) and initially food restricted to no less than 85% free feed weight. Rats were then subsequently fed 15–20 g of Purina laboratory chow each day. Procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and in accordance with the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee.

### Apparatus

Eight behavioral chambers (43 x 43 x 53 cm) housed in sound-attenuating cubicles (Med Associates, St. Albans, VT) were used. One side of each chamber contained two retractable levers with a cue-light above each and a food/water receptacle positioned equal distance between the levers. The opposite wall contained a nosepoke device with a houselight and speakers for tones and white noise mounted above it. The floor of each chamber was comprised of evenly spaced stainless steel bars (0.5cm dia, 1.5 cm apart). Cocaine infusion was controlled via a motor-driven syringe pump (Med Associates), and tubing was tethered using a counterweighted arm to provide for animal mobility. During tasks that involved food pellets, the nosepoke was covered with a plate to restrict access to it. During self-administration, the plate was removed and the levers were retracted. Operant chamber function and data input were controlled by MED-PC (Med Associates, St. Albans, VT).

### Surgery

Rats were anesthetized with a mixture of 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine and implanted with a chronic indwelling catheter (Access Technologies, Skokie, IL) into their right jugular vein under aseptic conditions. Implantation of catheters into the jugular vein is routinely completed in our laboratory and described previously (Carelli et al., 2000; Sadoris et al., 2016).

### Behavioral Procedures

All rats underwent extensive behavioral procedures conducted in consecutive phases. The timeline for behavioral training and description of the requirements of each phase are summarized in Fig. 1a, and described in detail below.

**Initial Behavioral Training (TR)**—Drug naïve rats were initially trained to press a lever for a sucrose pellet (45 mg, Purina TestDiet, Richmond, IN) with levers counterbalanced as left or right between animals. Upon obtaining 50 pellets for two consecutive days, rats were trained to lever press during a 5 s cue period in order to obtain reward; pressing outside of the 5 s cue window resulted in an error. After animals completed 2 consecutive sessions with at least 50% accuracy, they began the titration task.

**Titration Task (TT)**—The TT task (100 trials; depicted in Fig. 1b) is composed of a pre-cue period, cue period, post-cue period, and intertrial interval (ITI; variable time, VT 4 s). The lever was extended for the duration of the trial. Once the lever was pressed (or if the ITI began without the animal making a response), it was retracted until the beginning of the next trial. If the animal pressed during the cue period (when the cue light was illuminated), the lever retracted and a sucrose pellet was delivered. If the subject pressed the lever either before or after the cue light illuminated, no sugar pellet was given. These ‘errors’ were signaled by a short white noise burst and a five second timeout. During each trial, the pre-cue period lasted VT 1s and the post-cue period was 2s. The duration of the cue period varied depending on the individual’s past performance on the task. If the previous trial was correct, the cue duration became 10% shorter. If the previous trial was incorrect, the cue duration became 10% longer. If the rat omitted a trial, the duration remained the same. For each session, the initial cue duration was set to the previous session’s final cue duration. The TT phase lasted until the animal either made 100 responses or 1 hour elapsed. To ensure that animals had sufficiently learned the TT before beginning the DT task, they had to have between 40 – 60% correct responses and had to meet criteria for stability in task performance. Stability was defined as no significant changes in cue duration or percent of correct responses over three consecutive days.

**Distress Tolerance Task (DT)**—Following acquisition of TT, the same drug naïve rats began the DT task. This task had similar structure to the TT, but was designed so that the cue duration became progressively shorter until it was almost impossible for the animals to obtain a correct response. The initial cue duration was tailored towards each animal’s ability in the TT, and equal to the average response latency on correct trials in the TT for each rat multiplied by two. Early in the DT task, incorrect responses had no effect on cue duration while correct responses rapidly and progressively decreased it according to the formula: Cue Duration = Previous Cue Duration \*  $0.9^n$ , where n = the number of correct responses obtained thus far by the rat. Rats could only obtain pellets during the first 20 responses (by the end of which the cue duration was typically < 250 ms) and were allowed a maximum of 5 pellets in the task. After 20 responses or 5 correct responses (whichever came first), all responses (correct or incorrect) decreased cue duration by 10%. Omissions did not affect cue duration.

Our goal with the DT task was to determine each rat’s ability to persist in lever pressing in the presence of ‘psychological’ distress (forced failure, negative feedback). Rats were considered to have quit the task (our dependent measure) when they obtained 5 consecutive omissions directly preceded by 3 omissions out of 5 trials (thus 8 omissions out of 10 trials total). This time was capped at 1 hr if the animal did not ever reach criterion.

**Self-administration (SA)**—After the DT task was completed, animals underwent surgery for intravenous catheterization and began the self-administration task one week later. Here, a nosepoke led to an intravenous infusion of 0.33 mg of cocaine (n = 14) or an equivalent volume of both saline (i.v.) and water (delivered to the water receptacle) (n = 7) coupled with a 30s houselight and tone compound stimulus. Nosepokes during this 30s period did not result in additional reinforcement. The rationale for water training was to keep the

degree of operant responding (# of nose pokes) for cocaine versus control rats similar. The self-administration task lasted for 6 hr/session for 14 days. A subset of the controls only underwent 2 hr access for water/saline. Other than the amount of water/saline administered, there were no significant differences between 2 and 6 hr water/saline rats; thus both groups were collapsed for all analyses unrelated to self-administration. Cocaine hydrochloride was obtained from the National Institute on Drug Abuse and dissolved in 0.9% saline.

**Extinction (EX; Day 1)**—After completion of the self-administration phase, rats underwent a 34 day drug abstinence period during which a number of additional behavioral measurements were obtained. On day 1 of abstinence, rats underwent a 2 hr extinction task which was identical to the self-administration procedure described above, but no reinforcers were delivered.

**Distress Tolerance (DT; Days 7 & 28)**—On days 7 and 28 of abstinence, rats were again tested on the DT task, using identical procedures as described above. Each DT test was preceded by 3 days on the TT.

**Extinction (EX; Day 30)**—On day 30 of abstinence rats underwent an additional 2 hr extinction phase, as described above.

**Self-Administration (SA; Day 35)**—Finally, 5 days following the day 30 extinction session, an additional 2 hr self-administration session was conducted, identical to the original self-administration sessions described above.

**Exclusions**—Some rats had to be excluded from part of the analysis. Two cocaine rats lost patency during the initial self-administration period and were excluded from all subsequent analyses. One cocaine rat died for reasons unrelated to the experiment during the abstinence phase. Additionally, three cocaine rats lost patency during abstinence and could not be tested on the final self-administration session. Five of the water/saline rats were not tested on the day 7 DT test or any of the extinction or self-administration behavior during abstinence. Finally, due to a program error, latencies to the first nose poke were not recorded in seven of the cocaine rats during extinction day 1.

## Data Analysis

**Distress Tolerance (Drug Naive)**—To establish that the DT task was more challenging than the TT we used t-tests to examine task differences in percent of correct responses and number of omissions.

**Relationship of DT to Motor Impulsivity**—To determine if DT was related to any other behavioral phenotype, Pearson correlations were calculated between DT and measures in the TT (cue duration, omissions, latency, and impulsivity; averaged over the 3 stable days before the DT task). Impulsivity was defined as number of early errors/(number of early errors + number of late errors) reflecting the proportion of early errors.

**Self-Administration**—The primary dependent measures for self-administration were mg/kg of cocaine self-administered (or ml of water for controls), latency to first nose poke,

and loading behavior. Loading behavior was defined as the number of rapid consecutive responses early in the session that were less than half of the inter-reinforcer interval for the entire session; this measure has been previously correlated with measures of motivation to acquire drug in our earlier work (Carelli and Deadwyler, 1996; Wheeler et al., 2008). To determine if 6 hr access to cocaine resulted in an escalation of cocaine intake as previously reported, we ran a 1-way ANOVA on the amount of cocaine consumed by rats across the 14 days of self-administration followed by Bonferroni post hoc tests. Pearson correlations examined if drug-naïve DT or cue duration predicted any behavior during self-administration (amount consumed, loading, and latency to first press), including the last 3 days when escalation of intake occurred. Finally, we determined if impulsivity interacted with the ability of DT to predict self-administration. Here, we used the moderation analysis macro PROCESS (Hayes, 2013), with drug naïve DT as the independent variable, self-administration behavior (amount consumed, loading, or latency to first press) as the dependent variables, and drug naïve impulsivity as the moderator.

**Distress Tolerance (Abstinent)**—To confirm that the DT task remained more challenging than the TT following abstinence, we examined task differences in percent of correct responses and number of omissions using t-tests. To determine test-retest reliability, Pearson correlations compared abstinent DT with drug naïve DT. A 2 x 2 mixed design ANOVA with Bonferroni post hoc tests was completed with Time (Drug-Naïve, 28 d of abstinence) and Drug (Cocaine, Water/Saline) as factors to examine the effect of cocaine on DT.

**Extinction**—The dependent measures for extinction were the number of nose pokes and the latency to first nose poke. A paired sample t-test was completed to compare the number of nose pokes during extinction after 1 d and 30 d of abstinence. To determine if DT predicted extinction behavior, Pearson correlations on these data were calculated. Finally, to determine if impulsivity influenced the ability of DT to predict extinction, we conducted moderation analyses, using DT (drug naïve, day 7, or day 28) as the independent variable, extinction behavior (number of nose pokes or latency after 1 or 30 d of abstinence) as the dependent variable, and impulsivity (drug naïve, day 7, or day 28) as the moderator.

**Self-administration (Day 35)**—Pearson correlations were calculated to determine if DT (drug naïve, day 7, or day 28) predicted self-administration. To determine if impulsivity influenced the ability of DT to predict self-administration behavior, we conducted moderation analyses, using DT (drug naïve, day 7, or day 28) as the independent variable, self-administration (amount consumed, loading, and latency to first press) as the dependent variable, and impulsivity (drug naïve, day 7, or day 28) as the moderator.

All statistical analyses were completed with SPSS version 23 and Graphpad Prism.

## Results

### Initial Behavioral Training

Rats learned to lever press for a sucrose pellet over  $6.14 \pm 0.49$  days, then to respond within a 5 s cue period with at least 50% accuracy after  $4.43 \pm 0.98$  days.

### Titration Task

Rats made  $49.61 \pm 0.49\%$  correct responses following  $14.81 \pm 0.87$  days of titration training. During the last 3 training days, responding was stable as indicated by consistent: 1) cue duration periods ( $1.75 \pm 0.17$ s), 2) percent correct responses ( $49.61 \pm 0.49\%$ ) and, 3) number of omissions ( $21.95 \pm 6.29$ ).

### Distress Tolerance (Drug Naïve)

Next, rats entered the DT phase, in which the cue duration became progressively shorter over the course of the session. Behavior reflected this increased difficulty, as rats had significantly fewer percent correct responses ( $t(20)=72.99$ ,  $p<0.001$ ; Fig. 2A) and more omissions ( $t(20)=14.33$ ,  $p<0.001$ ; Fig. 2B) than during the TT. All rats stopped responding before one hour had elapsed, with some quitting quickly and others taking almost the entire hour (range of time to quit: 16.63–59.32 min, Fig. 2C).

Importantly, since baseline omission levels during the TT could directly influence our DT measure, we ran a correlation between baseline omissions in the TT and subsequent DT, but found no relationship ( $r^2 = 0.07$ ,  $p=0.242$ ). Thus, baseline motivation for sucrose likely had little impact on DT. Finally, we found no relationships between DT and baseline cue duration ( $r^2=0.08$ ,  $p=0.212$ ), latency ( $r^2=0.05$ ,  $p=0.318$ ), or proportion of early errors ( $r^2=0.04$ ,  $p=0.413$ ), suggesting that DT was unrelated to task ability, reaction speed, or impulsivity.

### Self-Administration (SA)

Next, animals underwent surgery and, one week later, SA training. Across the last three days of training, rats ( $n=12$ ) showed stable responding, completing  $74.55 \pm 5.29$  nosepokes with a mean ITI of  $5.18 \pm 0.47$  min (total amount of cocaine consumed was  $55.78 \pm 3.57$  mg/kg/d). Characteristic of 6 hr access to cocaine, rats escalated intake across the 14 days of SA ( $F(13,143)=3.796$ ,  $p<0.001$ ; Bonferroni post hoc tests show days 9–12 significantly greater than day 1; Fig. 3B). Animals self-administering water and saline ( $n=7$ ) also showed stable behavior, completing  $25.67 \pm 3.09$  nosepokes (2 hr session: mean ITI;  $7.52 \pm 2.44$  min), and  $83.50 \pm 8.50$  nosepokes (6 hr session; mean ITI;  $3.91 \pm 0.27$  min). A major goal of this study was to examine if drug naïve DT predicted cocaine seeking or taking behavior. Figure 3A shows where comparisons were made in our procedural timeline. Regression analysis showed that drug naïve DT did not predict cocaine intake or loading across all sessions, or over the last 3 days of SA (all  $p$ 's  $> 0.05$ ). However, drug naïve low DT did predict shorter latency to first response over the last 3 days of SA ( $r^2=0.35$ ,  $p=0.041$ ; Fig. 3C). Additionally, drug naïve impulsivity and cue duration both did not predict any element of SA, and no interactions between DT and impulsivity were significant (all  $p$ 's  $> 0.05$ ). Finally, neither drug naïve DT nor impulsivity predicted escalation of intake ( $p$ 's  $> 0.05$ ). Collectively, these data suggest that rats with low basal DT may have increased motivation to initiate drug-taking behavior following escalation of cocaine SA.

### Extinction (Day 1)

To determine if DT predicted drug-seeking, cocaine rats underwent a 2 hr extinction session on the first day of abstinence, making  $29.82 \pm 3.96$  responses with  $1.61 \pm 0.56$  min latency

to the first response. Drug naïve DT did not predict the number of responses during the task ( $r^2=0.18$ ,  $p=0.176$ ), nor was there an interaction with drug naïve impulsivity, indicating that drug naïve DT does not predict drug-seeking at the beginning of abstinence.

### Distress Tolerance (Days 7 & 28)

DT was again measured on days 7 & 28 of abstinence, and each DT test was preceded by three days on the TT. Similar to drug naïve TT and DT, rats showed evidence of the increased difficulty on the DT task, with the DT task associated with significantly fewer percent correct responses (day 7:  $6.33 \pm 0.49\%$  vs.  $46.39 \pm 1.57\%$ ,  $t(11)=29.13$ ,  $p<0.001$ ; day 28:  $8.62 \pm 0.89\%$  vs.  $49.54 \pm 0.45\%$ ,  $t(15)=52.22$ ,  $p<0.001$ ) and more omissions (day 7:  $119.83 \pm 14.34$  omissions vs.  $16.67 \pm 7.27$  omissions,  $t(11)=10.00$ ,  $p<0.001$ ; day 28:  $167.94 \pm 10.83$  omissions vs.  $21.99 \pm 6.05$  omissions,  $t(15)=10.72$ ,  $p<0.001$ ). Animals again exhibited high individual variability during both test days (day 7, range of time to quit: 12.41 – 59.80 min; day 28, range of time to quit: 3.11 – 42.29 min). Additionally, the DT task displayed good test-retest reliability, since drug-naïve DT correlated with DT after 28 days ( $r^2=0.38$ ,  $p=0.006$ ) of abstinence and had a trend to correlate after 7 days of abstinence ( $r^2=0.23$ ,  $p=0.083$ ).

One objective of the study was to determine if cocaine SA altered subsequent DT. Using a 2x2 mixed design ANOVA with time (drug-naïve, 28 d of abstinence) and drug (cocaine, water/saline) as the factors, we found a main effect of time ( $F(1,16)=20.32$ ,  $p<0.001$ ), indicating that all rats decreased DT across test days (drug naïve DT:  $30.97 \pm 2.07$  min, day 28 DT:  $21.52 \pm 2.88$  min), independent of drug condition (Fig. 4A). However, there was no main effect of drug ( $F(1,16)=2.10$ ,  $p=0.167$ ) and no time x drug interaction ( $F(1,16)=0.23$ ,  $p=0.639$ ). The latter suggests that a history of cocaine SA had no unique effect on subsequent DT on day 28. Next, we examined if the change in DT in each group (i.e., Day 28 DT - drug naïve DT) correlated with how much cocaine or water was consumed. Here, we found that within cocaine rats, greater cocaine consumption during SA (average of days 1–14) significantly correlated with a greater decrease in DT on day 28 ( $r^2=0.46$ ,  $p=0.022$ ; Fig. 4B), suggesting that the amount of cocaine consumed had a negative effect on DT. This correlation was not significant for water rats ( $r^2=0.00$ ,  $p=0.944$ ; Fig. 4C).

### Extinction (Day 30)

A second 2 hr extinction session was administered on the 30<sup>th</sup> day of abstinence (Fig. 5A). Here, rats with a history of cocaine SA showed an ‘incubation of craving’ effect, exhibiting a significant increase in the number of nose pokes compared to extinction after 1 day of abstinence ( $t(10)=2.95$ ,  $p=0.014$ , see Fig. 5B). Next, we determined if DT (drug naïve, day 7, or day 28) would predict the heightened drug-seeking behavior during extinction seen after 30 days of abstinence. We found that neither drug naïve nor day 7 DT predicted number of nose pokes or latency during extinction (all  $p$ 's > 0.05). However, low DT after 28 days of abstinence did have a trend to predict the high number of nose pokes during extinction ( $r^2=0.28$ ,  $p=0.092$ , see Fig. 5C).

Interestingly, there was an interaction between DT (day 28) and impulsivity (days 25–27) that significantly predicted the number of extinction nose pokes (day 30). Specifically, the



interactive effect of impulsivity and DT on extinction responses was estimated with an OLS regression model, with impulsivity as the moderator. The interaction term was significant ( $\beta = 15.150$ ,  $SE = 3.611$ , 95%  $CI$ : 6.606–23.694,  $p = 0.004$ ). To understand the nature of this moderation, conditional effects (“simple slopes”) of DT on extinction nose-pokes was estimated for low and high impulsivity ( $\pm 1$  SD). As illustrated in Figure 5D, high DT was associated with fewer extinction nose-pokes among rats with low ( $\beta = -2.979$ ,  $SE = 0.582$ , 95%  $CI$ :  $-4.357$  –  $-1.602$ ,  $p = 0.001$ ), but not high ( $\beta = 0.983$ ,  $SE = 0.717$ , 95%  $CI$ :  $-0.715$  –  $2.680$ ,  $p = 0.213$ ), impulsivity. These data suggest that, following extended abstinence, animals with high DT were protected against elevated drug-seeking behavior, but only if they also displayed low impulsivity. Importantly, impulsivity alone did not predict the number of nose-pokes during extinction ( $r^2 = 0.00$ ,  $p = 0.869$ ). We also found a significant interactive effect of impulsivity (average of days 4–6) and DT (day 7) on the latency to first press for extinction ( $\beta = 0.374$ ,  $SE = 0.116$ , 95%  $CI$ :  $0.996$  –  $0.648$ ,  $p = 0.015$ ). Upon examination of conditional effects of DT on extinction latency, we found that low DT predicted short latency to press in animals with high ( $\beta = 0.075$ ,  $SE = 0.024$ , 95%  $CI$ :  $0.017$  –  $0.132$ ,  $p = 0.018$ ), but not low ( $\beta = -0.040$ ,  $SE = 0.026$ , 95%  $CI$ :  $-0.100$  –  $0.021$ ,  $p = 0.165$ ), impulsivity. This suggests that rats with low DT and high impulsivity in early abstinence may more quickly initiate drug-seeking behavior after extended abstinence.

**Self-Administration (Day 35)**—A final 2 hr self-administration session occurred on the 35<sup>th</sup> day of abstinence. Here, rats self-administered  $10.59 \pm 1.83$  mg/kg of cocaine during the session (ITI =  $6.76 \pm 0.81$  min). As illustrated in Figure 6A, we determined if following extended abstinence, DT (drug naïve, day 7, day 28) would predict cocaine SA (Day 35). Neither drug naïve nor day 7 DT predicted amount of cocaine administered, loading or latency to first press (all  $p$ 's  $> 0.05$ ). However, DT after 28 days of abstinence significantly predicted loading during the task ( $r^2 = 0.54$ ,  $p = 0.038$ , Fig. 6B) and had a strong trend to predict the amount of cocaine self-administered ( $r^2 = 0.49$ ,  $p = 0.052$ , Fig. 6C). Impulsivity (drug naïve, days 4–6, days 25–27) did not predict SA, loading or latency on day 35 (all  $p$ 's  $> 0.05$ ). These data suggest that, following extended abstinence, animals with lower DT rapidly load up more drug and have greater drug consumption overall.

## Discussion

We developed a novel rodent model of DT based upon a human model, the Computerized Paced Auditory Serial Attention Task (PASAT-C, Lejeuz et al., 2003). Similar to human laboratory paradigms, DT was defined in our rat model as the amount of time it took the animal to continue to seek reward, while experiencing ‘psychological’ distress, before quitting the task. Animals exhibited a wide range of DT, with some rats quitting quickly and others nearly reaching the one hour time limit. We assessed the relationship of DT with cocaine seeking and taking both before and after one month of abstinence from SA. To build on existing human work with current substance users, we were particularly interested in examining the unique effects of drug naïve DT and post cocaine self-administration DT on cocaine seeking and taking. We found that drug naïve DT did not predict cocaine seeking and taking. However, low DT measured after abstinence from cocaine SA significantly predicted high levels of early session cocaine taking. Additionally, high DT measured after

abstinence protected against high cocaine seeking, but this protective effect was blocked in rats with high impulsivity.

Our primary finding of interest was that animals with low DT following cocaine SA exhibited heightened cocaine seeking and taking after a period of extended drug abstinence (30–35 days). This result is consistent with several human studies showing that substance users with low DT have a high rate of treatment dropout and relapse (Brown et al., 2002; Brandon et al., 2003; Daughters et al., 2005a,b; Brown et al., 2009; Strong et al., 2012). However, our findings also demonstrate the important role that extended drug abstinence plays in the relationship between DT and substance use. Previous reports showed extended abstinence from cocaine leads to an ‘incubation of craving’ which results in greater cocaine seeking (Grimm et al., 2001; also replicated here) as well as a host of molecular and neural adaptations in corticolimbic circuitry (Grimm et al., 2003; Lu et al., 2003; Lu et al., 2004). It is possible that our measurement of DT after extended abstinence is capturing individual differences in these effects, with rats demonstrating low DT experiencing higher incubation of craving. As such, it may be fruitful to investigate the differences in neural adaptation between high and low DT animals after incubation of craving.

DT’s ability to predict relapse and treatment dropout is thought to reflect how much a given individual can tolerate the distress associated with drug abstinence (Magidson et al., 2013). This is in line with negative reinforcement theories of addiction (Baker et al., 2004; Koob, 2013), which suggest that the heightened distress associated with drug withdrawal/abstinence drives an individual to seek and consume drug to alleviate that distress. These theories suggest that chronic drug use alters the hedonic set point of the brain by upregulating “antireward” brain systems, thereby requiring a greater amount of reward (natural or drug) to elicit the same response (Koob, 2013). It may therefore be valuable to investigate the effects of downregulating these systems (e.g. by blocking kappa opioid receptor function in reward circuitry, or corticotrophin-releasing factor receptor function in the extended amygdala) on DT.

We also found that motor impulsivity significantly influenced the role of DT in cocaine seeking (but not taking). Specifically, high DT after extended abstinence protected against elevated drug seeking, but high impulsivity was able to overcome this protective effect. Furthermore, low DT measured during early abstinence predicted faster latency to initiate cocaine seeking, but only in highly impulsive rats. Importantly, impulsivity alone did not predict drug seeking and taking, nor were DT and impulsivity related, collectively providing further evidence for the specificity of these constructs (Daughters et al., 2005a; Kiselica et al., 2015; however note that motor impulsivity in a 5-choice task does predict drug seeking and taking; Belin et al., 2008; Dalley et al., 2007). The results suggest that under psychologically distressing conditions, one’s ability to persist in goal-directed behavior is influenced by levels of disinhibition, with a combined low DT and high impulsivity presenting heightened risk for drug seeking.

In addition to determining DT’s predictive capacity, we investigated if a history of cocaine SA altered DT. Previous research has shown that cocaine history can disrupt goal-directed behaviors, and neural mechanisms underlying associative learning and decision making

(Stalnaker et al., 2007; Mitchell et al., 2014; Saddoris & Carelli, 2014; Saddoris et al., 2016). A history of cocaine SA had no unique effect on DT when compared to a history of water/saline self-administration. However, within cocaine rats, greater amounts of cocaine SA did correlate with a steeper decrease in DT, while among water rats there was no relationship with water/saline self-administration (although the latter had a relatively small sample size). Nevertheless, these findings are in line with previous research reporting a relationship between DT and degree of substance use (Daughters et al., 2016; Quinn et al., 1996).

In summary, using a translational approach, we developed a rodent model of DT to examine the relationships between aspects of DT and cocaine self-administration. Our results corroborate features of DT reported in human addicts and support our paradigm as a model for DT and drug relapse in rodents. It is important to note however that our primary task measure, DT, is not easily dissociated from other measures such as attentional ability or cognitive effort (e.g. Cocker et al., 2012), and future studies should investigate the relationships between these constructs. Nevertheless, our work sets the foundation for additional studies that can be designed to investigate the neural underpinnings of DT.

## Acknowledgments

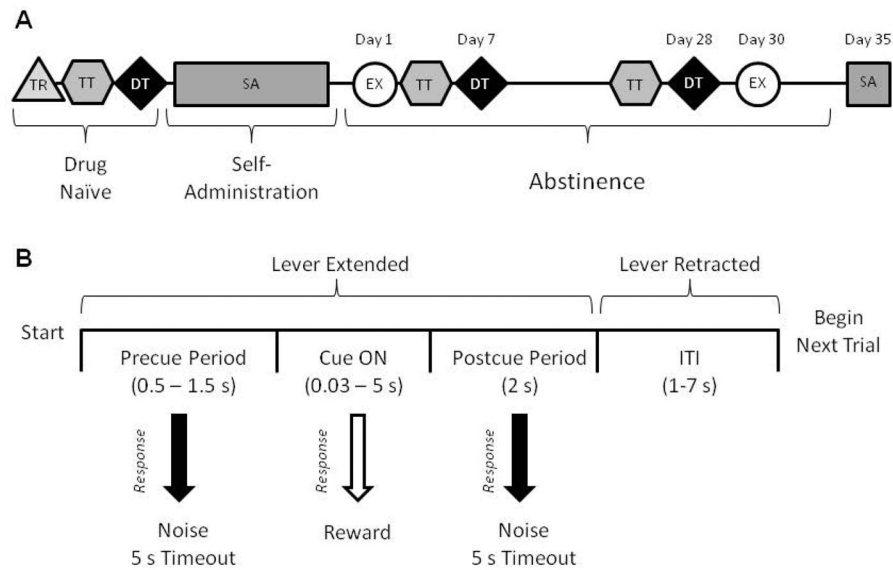
The work was supported by National Institute on Drug Abuse Grant DA014339 to RMC. We thank Dominic Stone and Xuefei Wang for technical assistance.

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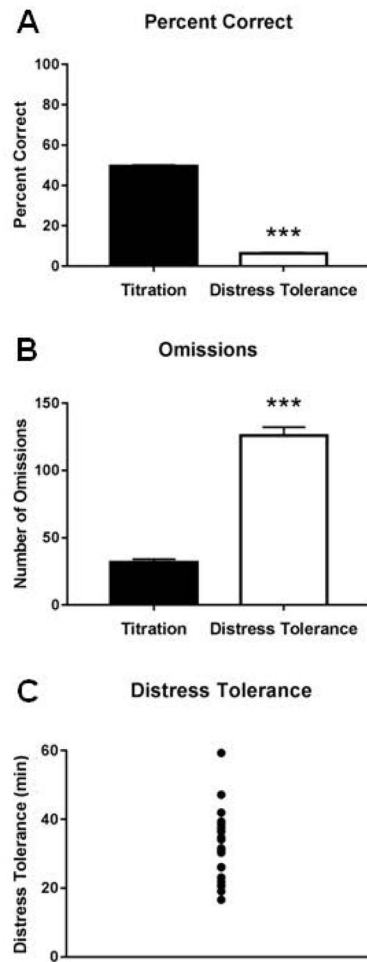
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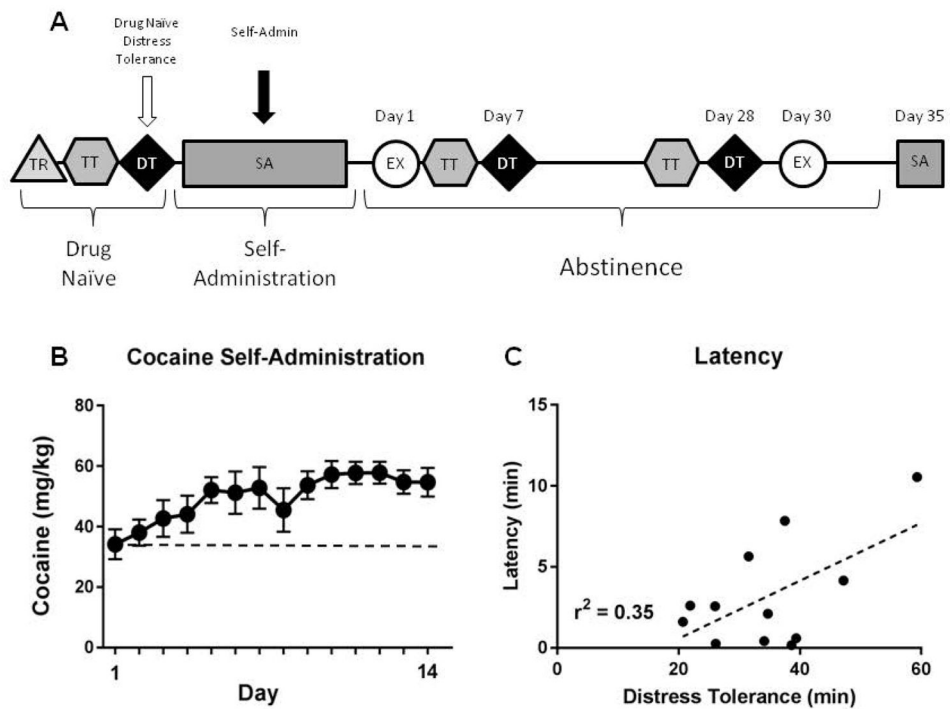
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**Figure 1.** Experimental timeline and schematic overview of distress tolerance task. A) Experimental timeline. TR = Initial Training; TT = Titration Task, DT = Distress Tolerance Task, SA = Cocaine Self-Administration, EX = Cocaine Extinction. See main text for details of task design. B) Schematic of a single distress tolerance trial. Responses during the cue period (Cue ON) were rewarded with a single sucrose pellet. Responses during the Precue or Postcue periods were considered ‘errors’ and were signaled by a noise and a 5 s timeout. The duration of the cue ON period titrated according to task performance in the Titration task, and progressively decreased in the Distress Tolerance task.

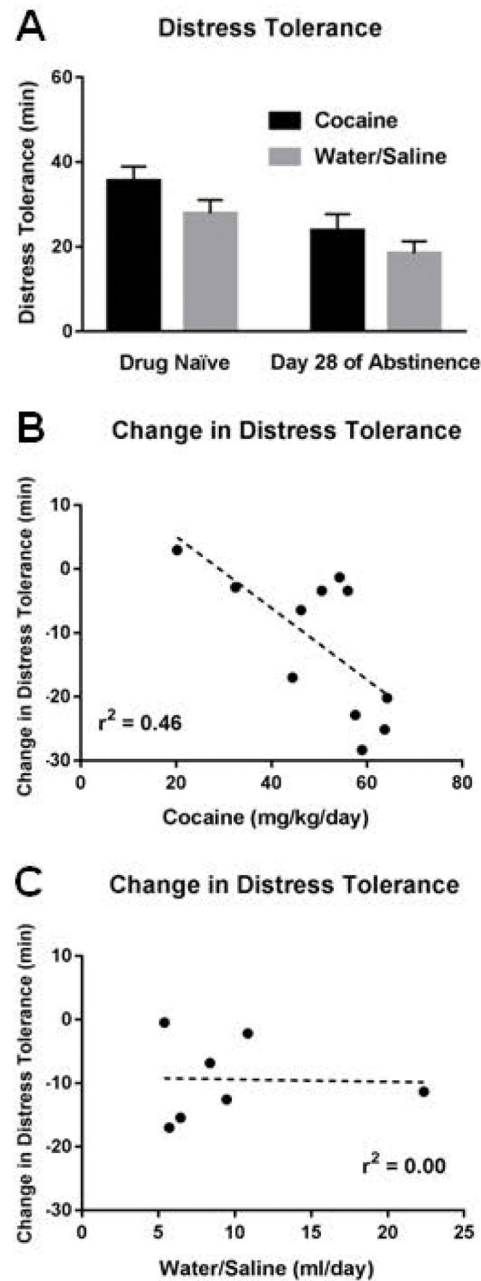


**Figure 2.** Behavior in the distress tolerance task. Rats exhibited significantly fewer percent correct responses (A) and more omissions (B) in the distress tolerance task than the titration task. C) Rats exhibited a wide range in how quickly they stopped responding (i.e., time to quit) in the distress tolerance task.

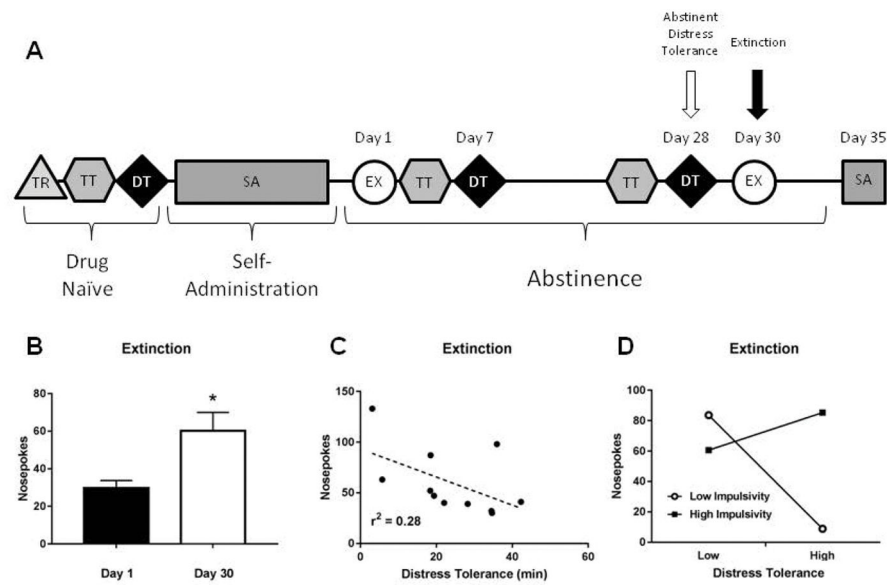


**Figure 3.** Relationship between drug naïve distress tolerance and self-administration behavior. A) Experimental timeline highlighting analysis time points. B) Animals significantly escalated cocaine intake over the 14 days of self-administration access. C) Drug naïve distress tolerance significantly predicted the latency to the first response of the last 3 days of cocaine self-administration.

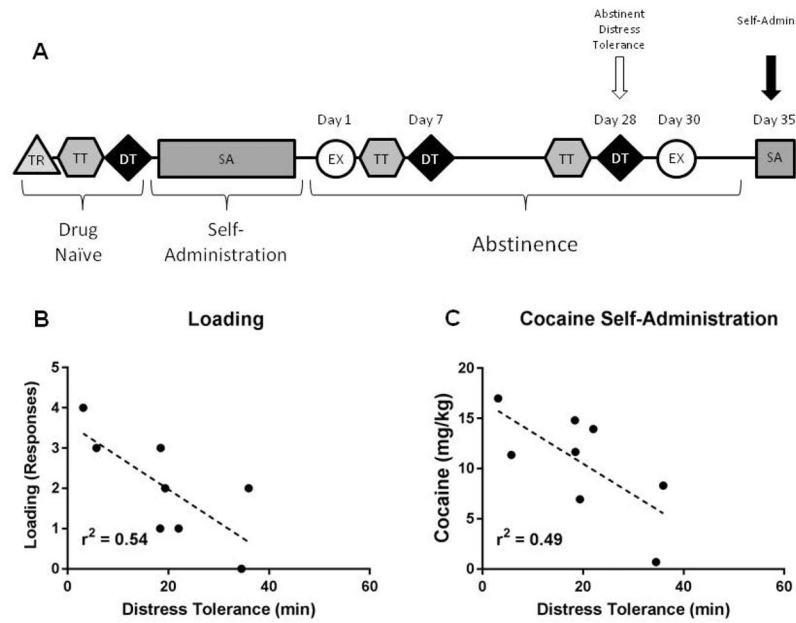




**Figure 4.** Relationship between cocaine self-administration and subsequent distress tolerance. A) All rats decreased distress tolerance across test days, independent of whether they had a history of water or cocaine self-administration. B) A history of greater cocaine consumption significantly correlated with a greater decrease in distress tolerance. C) A history of water consumption did not correlate with a decrease in distress tolerance.



**Figure 5.** Relationship between distress tolerance following abstinence and subsequent drug seeking behavior during extinction. A) Experimental timeline highlighting analysis time points. B) Rats exhibit ‘incubation of craving’ (i.e., increased drug seeking) following 30 days of drug abstinence, C) Distress tolerance after abstinence had a trend to predict number of responses under extinction. D) There was a significant interaction between distress tolerance and impulsivity: In animals with low impulsivity, low distress tolerance significantly predicted more responses under extinction. No such relationship was seen in animals with high impulsivity.



**Figure 6.** Relationship between distress tolerance following abstinence and subsequent drug taking behavior during self-administration. A) Experimental timeline highlighting analysis time points. Distress tolerance after abstinence B) significantly predicted loading behavior and C) had a trend to predict amount of cocaine self-administered.