# EXPLORING THE EFFECTS OF A CORTICOTROPIN RELEASING FACTOR (CRF) RECEPTOR ANTAGONIST ON HABIT EXPRESSION

by

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*To my grandfather and my aunt who are always there for me with knowing smiles*

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# ABSTRACT

Some individuals with alcohol use disorder (AUD) continue to drink because they have developed a habit in which they are not considering the consequences of their actions. Habitual actions persist despite changes in reward and are often studied using devaluation procedures. Stress hormones, such as corticotropin releasing factor (CRF), have been linked to AUD when examining binge-like drinking and withdrawal in rodents. Stress has been examined in the switch from goal-directed to habitual behavior, and CRF has often mimicked the effects of stress exposure. This study looked at the possible direct effects of CRF on habit expression in rats using an operant paradigm. Finding possible novel mechanisms of habit could create an avenue for future novel treatment options. Female and male Long Evans rats were trained on a variable interval schedule using sucrose as a reward. Rats then underwent devaluation procedures including both sensory-specific satiety and conditioned taste aversion (CTA) to test for habitual behaviors. Prior to an extinction session post-CTA, animals were treated with either 20 mg/kg R121919, a CRF1 receptor antagonist, or vehicle. A second extinction session was conducted where animals received the alternative treatment. Lever presses were recorded as a measure of goal-directed or habitual behavior. Sensory-specific satiety devaluation tests revealed that animals were not sensitive to devaluation. This was further supported by both post-CTA extinction sessions. R121919 had no effect on lever pressing in either devalued or valued groups. Further research is needed to explore how a CRF receptor antagonist may affect habit formation or the transition from goal-directed to habit behaviors. Future studies should also examine any possible interaction effects CRF may have with alcohol or stress on habitual behaviors.

# INTRODUCTION

## General Introduction

Alcohol use disorder (AUD) is characterized by persistent excessive alcohol use despite negative consequences (American Psychiatric Association, 2013). AUD affects over 76 million people worldwide (Labots et al., 2018) and over 16 million people in the United States (NIAAA, 2018). An individual who initially sought alcohol for its rewarding properties can begin to demonstrate inflexible behaviors (Everitt & Robbins, 2016). Inflexible behaviors can also be classified as stimulus-response associations (habits) and are often demonstrated by the reward being devalued and the response remaining the same (Barker et al., 2015; Everitt & Robbins, 2005). This study specifically examined a possible novel mechanism of action involved in habitual behavior.

Corticotropin releasing factor (CRF) is a 41 amino acid peptide synthesized in the paraventricular nucleus of the hypothalamus that plays a role in stress response (Bale & Vale, 2004; Vale, Spiess, Rivier, & Rivier, 1981). In the hypothalamus, CRF stimulates the release of adrenocorticotropin hormone (ACTH) which regulates the release of glucocorticoids and mineralocorticoids via the hypothalamus-pituitary-adrenal (HPA) axis (Bruijnzeel & Gold, 2005). Extrahypothalamic CRF has been implicated in drug-seeking reinstatement (Schwabe, Dickinson, & Wolf, 2011) and is often discussed in the context of alleviating the negative effects of drug withdrawal (Koob & Volkow, 2010). For example, CRF receptor antagonists have been able to decrease stress-induced reinstatement of alcohol seeking in a footshock paradigm (Le et al., 2000) and CRF is associated with increased stress responsiveness (Koob & Zorrilla, 2010). It is known that stress can be linked to increased habit memory (Goodman, Leong, & Packard, 2015) and that stress may increase habitual responding due to a disruption in reward processing (Bogdan, Santesso, Fagerness, Perlis, & Pizzagalli, 2011). However, few studies have linked the possible effects of CRF on habit in animal models, and none have examined the effects of CRF antagonists on habit expression.

## Corticotropin Releasing Factor

Extrahypothalamic CRF has often been studied in relation to alcohol withdrawal. Koob and Volkow (2016) explain that during withdrawal, CRF increases can be seen in the extended amygdala and that common anxiety and stress behaviors can be decreased with CRF receptor antagonists. The two types of CRF receptors, CRF1 and CRF2, are G-protein coupled receptors (Bruijnzeel & Gold, 2005). Generally, CRF1 receptors are thought to be linked to stress responsiveness and CRF2 receptors are associated with decreases in feeding behavior (Koob & Zorrilla, 2010). There is evidence that CRF2 receptors do a play a role in stress response and that it is opposite in nature compared to CRF1 receptors (Robinson, Perez-Heydrich, & Thiele, 2019). Although CRF has a tenfold higher binding affinity for CRF1 receptors, where it stimulates stress response, urocortin II (UcnII) and UcnIII have a higher affinity for CRF2 receptors (Bale & Vale, 2004). UcnI, UcnII, and UcnIII, are ligands part of the broader CRF family system and may contribute to some of the effects seen in CRF studies. CRF1 receptor antagonists have decreased alcohol self-administration in compulsively seeking and dependent animals (George, Le Moal, & Koob, 2012; Gilpin, Richardson, & Koob, 2008), reduced cocaine self-administration (Corominas, Roncero, & Casas, 2010), and mediated alcohol psychomotor sensitization (Pastor et al., 2008). Some studies have shown a reduction in drinking in non-dependent, non-alcohol preferring rodents (Cippitelli et al., 2012; Simms, Nielsen, Li, & Bartlett, 2014). These results are in direct contrast with data in alcohol-preferring rodent lines that found no differences in alcohol consumption in non-dependent animals (Gilpin et al., 2008; Sabino, Kwak, Rice, & Cottone, 2013). Treatment with R121919, a CRF1 receptor antagonist, either directly into the amygdala or systemically, reduces operant self-administration and avoidant behavior after predator odor exposure (Weera, Schreiber, Avegno, & Gilpin, 2020). The mechanisms between CRF and self-administration have not been completely revealed. In addition, there is some discrepancy in the literature regarding if animals have to be alcohol dependent or not before CRF receptor antagonists become effective at reducing self-administration.

Brain regions such as the medial prefrontal cortex (mPFC) have been implicated in drug-seeking which is an important aspect of AUD. Hupalo, Bryce, et al. (2019) found that CRF in the mPFC impaired working memory and also may impact frontostriatal circuits in male rats. In mice, it was found that an injection of a CRF1 receptor antagonist into the mPFC reduced

ethanol and sucrose consumption in a drinking-in-the-dark (DID) paradigm while a CRF2 receptor antagonist reduced only ethanol consumption (Robinson et al., 2019). CRF and CRF receptors influence multiple brain regions associated with AUD. As stated previously, a bulk of the literature studying alcohol and CRF has focused on withdrawal; however, there is evidence that CRF may be impacting multiple behaviors in rodents in regard to alcohol seeking and consumption. The use of CRF receptor antagonists, particularly CRF1, help explain roles the CRF system plays in integrating stress, anxiety, and addiction and are a useful tool to help discover other implications for CRF and behaviors associated with addiction, such as habit.

## Habit Formation: Alcohol Use Disorder

Alcohol seeking behavior may become habitual for those who continue to drink even when they no longer desire it (Barker & Taylor, 2014) and these habits persist despite changes in reward (Hopf & Lesscher, 2014). This transition signals a shift from goal-directed behavior (response-outcome) in which an action depends on a reward to habitual behavior (stimulus-response) in which an action is unaffected by changes in reward (Dickinson, 1985; Houck & Grahame, 2018). In rodents, devaluation of a reward can be done by giving free access to the reward prior to testing or using lithium chloride to produce a conditioned taste aversion (CTA) (Corbit & Janak, 2016; Dickinson, Wood, & Smith, 2002). In humans, there can be reduced behavioral flexibility with AUD, supporting the idea that habitual behavior is associated with addiction (McKim, Shnitko, Robinson, & Boettiger, 2016). The shift from reward-seeking in the early stages of addiction to habitual actions is due to mechanisms in the dorsal striatum (Koob & Volkow, 2010) and the corticostriatal circuit is involved in habit formation and instrumental leaning (Barker et al., 2015; Gremel & Costa, 2013). Rats with lesions to this area remain goal-directed and sensitive to devaluation while sham lesioned rats did not (Balleine & O'Doherty, 2010). Yin, Knowlton, and Balleine (2004) also found that rats given dorsolateral striatal lesions reduced their performance for a devalued reward while sham lesioned rats performed the same for both valued and devalued rewards. This gives further evidence that dorsolateral striatal lesions obstruct stimulus-response habit leaning, but goal-directed behavior remains the same.

Animal models of habit formation that use alcohol as a reinforcer are a useful tool in studying behavior and possible causes of behavior of individuals with AUD in addition to possible treatments (O'Tousa & Grahame, 2014). Animals trained in an operant paradigm for a

short period of time will become sensitive to changes in outcome, however, following extended training, animals are no longer sensitive to these changes (Corbit, Nie, & Janak, 2012). Operant responding has also been shown to persist longer under extinction trials if a variable interval (VI) schedule is used during training (McKim et al., 2016). Therefore, longer consistent training, or over-training, using a VI schedule is more likely to form habits in rodents. Tricomi, Balleine, and O'Doherty (2009) found that similar to rodents, over-training humans can also create habitual actions as participants responded similarly for a devalued snack as a non-devalued snack.

## Habit Formation: Sex Differences

Operant training paradigms are useful in studying habitual behavior that relies on dopamine pathways in the dorsal striatum. Estrogen has been shown to moderate dopamine function (Korol, 2004) and release in the striatum (Becker, 1999) and sex differences have been seen in striatal organization (Becker, 1999). Some evidence has also suggested that when given sucrose pellets as a reward, female rats develop habitual operant responding behavior more rapidly than males (Schoenberg, Sola, Seyller, Kelberman, & Toufexis, 2019). In mice, conflicting sex differences have been found in the rate of habit formation, possibly due to food versus alcohol being used as a reinforcer (Barker, Torregrossa, Arnold, & Taylor, 2010; Quinn, Hitchcott, Umeda, Arnold, & Taylor, 2007). Considering the import role of dopamine function and release in drug use and habit formation (Schwabe, Dickinson, et al., 2011), and general sex differences seen in other phases of addiction (Becker & Hu, 2008), it is important to study possible differences in habitual responding between female and male rodents because striatal function may differ.

## CRF, Stress, and Habit

Alcohol and stress can lead to prefrontal cortex dysfunction which can then cause an automatic habitual response to replace goal-directed behavior (Blaine & Sinha, 2017). Stress, and hormones within the stress system, could affect multiple memory systems. For example, CRF has been shown to alter dendritic spines in the hippocampus possibly leading to a disruption of spatial memory (Chen et al., 2010). CRF1 receptors could also impact reinforcing effects of alcohol as the CRF1 receptor antagonist MPZP dose dependently reduced operant responding in

dependent alcohol preferring (P) rats (Gilpin et al., 2008). There may be sex differences in rodent models of stress and CRF as female rodents exhibit greater stress response compared to males (Becker & Koob, 2016). Female CRF1 receptor knockout mice in a conditioned place aversion following morphine withdrawal showed a higher decrease in ACTH compared to males and there is a potential sex difference in the learning and recall of aversive stimuli (Garcia-Carmona, Baroja-Mazo, Milanes, & Laorden, 2015).

Research has demonstrated that acute stress after devaluation makes humans more likely to show habitual over goal-directed behaviors possibly due to noradrenergic activity or other stress-related hormones (Schwabe, Dickinson, et al., 2011; Schwabe, Hoffken, Tegenthoff, & Wolf, 2011; Schwabe & Wolf, 2010). Rats showed better retention of procedural training after glucocorticoid administration into the dorsal striatum (Quirarte et al., 2009). Although not CRF specific, this provides evidence of stress systems having an effect on habit formation and procedural learning and memory. Lemos, Shin, and Alvarez (2019) found that a bath application of CRF increased firing of cholinergic interneurons in the striatum which express CRF1 receptors. The authors hypothesized that CRF could affect dopamine transmission through activation of muscarinic acetylcholine receptors or by direct activation of CRF receptors on dopamine terminals. There is already evidence for a role of the CRF system on habitual behaviors when examining nicotine sensitization and altered CRF expression in the dorsal striatum, hippocampus, and prefrontal cortex (Carboni, Romoli, Bate, Romualdi, & Zoli, 2018). CRF could have a broad role in stimulus-response associations and procedural learning and memory which may have larger implications for exploring possible causes and treatments of addiction.

## Hypotheses

CRF has not been directly studied in relation to habit and alcohol. CRF antagonists have attenuated rodent intake of palatable foods in home cages and after yohimbine-induced reinstatement (Cottone et al., 2009; Ghitza, Gray, Epstein, Rice, & Shaham, 2006). These findings suggest that CRF may play a role in responding for natural rewards, such as sucrose, when given intermittent access. This study examined CRF and the effect it may have on habit expression in rats by using systemic injections of the CRF1 receptor antagonist R121919. I hypothesized that R121919 would attenuate habit expression. Based on conflicting results of

females and males' performance in habitual paradigms and also sex differences within stress systems, I hypothesized that there is a strong possibility of a sex difference and that the effects of R121919 would be greater in females.

# METHODS

## Subjects

24 female and 24 male adult Long Evans rats were used. Animals were on a 12hr light/dark cycle (lights on 07:00-19:00) and had *ad libitum* access to food. Long Evans were chosen based on previous studies that found successful habitual behavior (e.g. Corbit et al., 2012; Nie & Janak, 2003; Vandaele, Pribut, & Janak, 2017). All animals were single-housed and handled for three days prior to any procedures. One female rat failed to train and was dropped from the study leaving a total of 47 animals included.

## Equipment

The operant chambers used are 30 x 30 x 24.5 cm from Med-Associates, East Fairfield, VT and are equipped with two retractable levers, an illuminated house light, and a retractable sipper tube with a stainless-steel double ball barring spout. Chambers are individually kept in sound-attenuating cabinets. Data from the operant chambers was collected using MED-PC software (Med-Associates).

## Drugs

Sucrose was used as a reinforcer in the chambers and mixed in water at concentrations of 1.5, 2, or 3%. Maltodextrin was used as a control reinforcer and mixed in water at concentrations of 1 or 2%. Lithium Chloride (LiCl) for CTA procedure was dissolved in sterile water and injected intraperitoneally (i.p.) at 95.25 mg/kg at a volume of 10 mg/ml. R121919 was dissolved in 20% 2-hydroxypropyl-beta-cyclodextrin (HPβCD; w/v in DI water) and injected subcutaneously (s.c.) at 20 mg/kg at a volume of 2 ml/kg 45 minutes prior to extinction trials (Sabino et al., 2013). Dosing was chosen based on previous work by Funk, Zorrilla, Lee, Rice, and Koob (2007) in which 20 mg/kg was used as the highest dose and reduced ethanol self-administration in dependent animals compared to vehicle treated animals. Vehicle injections were 20% HPβCD using the same volume, route, and time schedule as R121919 injections.

## Procedure

### Training

Animals were shaped with only the active lever present using sucrose as a reinforcer on a fixed ratio (FR)1 schedule in one 45 minute-session per day. Animals were given free access to the sipper tube for approximately 200 licks on the first day of training before beginning to be shaped on an FR1 schedule. 1.5% sucrose was used for the first 7 days of FR1 training, 2% sucrose was used for 3 additional days, until a final concentration of 3% was used as a reinforcer for the duration of the study. The inactive lever was introduced one week after training began. For the first five days of training animals were water-deprived and given access to home cage water for 60 minutes per day following operant-training sessions. They were then given normal access to home cage water for 12 days. Following normal water access, animals were water-restricted throughout the rest of the study where water was pulled between 09:00-10:00 and returned after operant sessions before the start of the dark cycle (19:00). Animals were given a total of 20 FR1 sessions before being moved to an increasing variable interval schedule (VI) consisting of one VI7, one VI15, and two VI30 sessions before moving to a VI60 schedule. Sensory-specific satiety devaluation was tested after 7 and 11 VI60 sessions. Conditioned taste aversion and additional extinction trials were tested after 15 VI60 sessions. A visual timeline for general procedures can be seen in Figure 1.

### Sensory-Specific Satiety Devaluation

On day 13 of training rats were given access to maltodextrin in their home cages to avoid neophobia. Animals were divided into devalued and valued groups based on operant responding on day 7 of VI60 sessions. Devalued animals received 45 minutes of free access to 3% sucrose (training reward) in their home cages and valued animals were given free access to maltodextrin (control reward). The first satiety devaluation procedure (after 7 VI60 days) used 2% maltodextrin and the second test (after 11 VI60 days) used 1% maltodextrin. Immediately following pre-feeding animals were placed in operant chambers for a 15-minute extinction trial in which no reward was delivered. On the second day of testing, animals were given free access to the alternate reward. Testing days one and two were separated by one day of normal VI60 training. This procedure was chosen based on previous satiety-induced devaluation procedures

that used sucrose as a training reinforcer (Corbit et al., 2012; Hay, Jennings, Zitzman, Hodge, & Robinson, 2013; Shillinglaw, Everitt, & Robinson, 2014; Vandaele, Pribut, & Janak, 2017) although some methodological changes were made (e.g. only using sucrose as a reinforcer). Prior to pre-feeding in the home cage animals received a s.c. injection of saline to acclimate animals to injections prior to drug delivery.

**Conditioned Taste Aversion**

Animals were divided into devalued and valued groups based on day 15 of VI60 operant responding. Devalued animals received 30 minutes free access to 3% sucrose (training reward) in their home cages immediately followed by an i.p. injection of LiCl. Valued animals received 30 minutes free access to 1% maltodextrin (control reward) followed by an i.p. injection of LiCl. All rats received at least three days of reward and LiCl pairings but could receive up to seven days. All animals met CTA criteria of a 75% decreased intake from baseline. After all CTA procedures animals were given one rest day before undergoing 15-minute extinction trial. 45-minutes prior to extinction animals received either an injection of R121919 or vehicle. A second extinction day was given in which animals were injected with the drug they had not received on day one.

**Analyses**

Active lever presses were the main measure of habitual responding in extinction trials after sensory-specific satiety devaluation and conditioned taste aversion. For sensory-specific satiety devaluations a 2 (order: devalued first vs. valued first) x 2 (sex: female vs. male) x 2 (day: devalued vs. valued) mixed methods ANOVA was used. Post CTA extinction trials were analyzed with a 2 (group: devalued vs. valued) x 2 (sex: female vs. male) x 2 (drug: R121919 vs. vehicle) between-subjects ANOVA. The two post-CTA extinction trials were compared using a mixed methods ANOVA comparing R121919 and vehicle as a within-subjects variable. Data were collapsed across variables where appropriate and follow-up simple effects tests were conducted on any significant interaction effects.

# RESULTS

## Operant Training

Active lever presses during training days are shown in Figure 2. Only the last 10 days of FR1 schedule are shown because access to the sipper tube after a lever response started at 30 seconds and then was progressively moved to 5 seconds based on individual animal's performance. It is important to note that on VI60-5 10 female rats' lever presses and intake were not recorded due to mechanical failure and so data points on that day represent the averages of fewer animals ($n$ = 37; 13 females, 24 males). For active lever presses, a mixed-effects model found a main effect of day, $F(2.06,91.75) = 28.91$, $p < .001$. Due to nearly non-existent responding, inactive lever data are not shown. Intake data was also recorded across training days (see Figure 3). A mixed-effects model found a main effect of day, $F(5.80,259.1) = 11.37$, $p < .001$, and sex, $F(1,45) = 7.68$, $p = .008$ where females consumed more sucrose than males.

## Sensory-Specific Satiety Devaluation One

A mixed methods ANOVA found no significant main effect of day, $F(1,43) = 0.09$, $p = .891$. There were also no main effects of sex, $F(1,43) = 0.53$, $p = .472$, or order $F(1,43) = 0.27$, $p = .607$. There was an interaction of day and order, $F(1,43) = 10.01$, $p = .003$, $\eta_p^2 = .189$. A split file comparison, collapsed across sex, found that animals who were devalued on day one had significantly more lever presses on devalued day ($M$ = 33.48, $SD$ = 26.98) compared to valued ($M$ = 27.26, $SD$ = 17.83), $t(22) = 2.28$, $p = .033$, $d = .68$ (see Figure 4A). Animals who were valued on day one did not have a significant difference in lever presses between devalued ($M$ = 30.63, $SD$ = 24.55) and valued ($M$ = 36.38, $SD$ = 25.35) days, $t(23) = 2.06$, $p = .051$. Normality tests revealed that assumptions were violated, and four outliers were identified. A similar pattern of results was found when outliers were removed ($n$ = 43), no main effects of day, sex, or order but a significant interaction of day and order. However, a split file follow-up t-test opposed earlier findings in which animals valued on day one had significantly more lever presses on valued day ($M$ = 31.77, $SD$ = 20.53) compared to devalued ($M$ = 25.64, $SD$ = 18.00), $t(21) = 2.56$, $p = .018$, $d = 2.42$ (see Figure 4B). No significant difference was found between valued ($M$ = 23.29, $SD$ = 12.60) and devalued ($M$ = 26.95, $SD$ = 16.84) days in animals that were devalued

on day one, $t(20) = 1.63$, $p = .119$. In an attempt to clear up the discrepancy a Wilcoxon Signed Ranks Test was ran comparing devalued and valued, with all animals included, still split based on order. The non-parametric test revealed an overall pattern that animals pressed more on day one of testing compared to day two. This test found that both groups were significantly different such that those that were devalued first had significantly more lever presses on devalued compared to valued, $Z = 1.98$, $p = .048$, and those that were valued on day one had significantly more lever presses on valued day compared to devalued, $Z = 2.36$, $p = .018$.

### Sensory-Specific Satiety Devaluation Two

Active lever presses were analyzed using a mixed methods ANOVA found no significant main effect of day, $F(1,43) = 2.28$, $p = .138$, devalued ($M = 24.15$, $SD = 19.97$) and valued ($M = 27.13$, $SD = 17.83$) days did not differ (see Figure 5A). There were also no main effects of sex, $F(1,43) = 0.14$, $p = .709$, or order, $F(1,43) = 0.10$, $p = .749$ and no significant interactions were found. Normality tests revealed that assumptions were violated, and one outlier was identified. A second ANOVA run with the outlier removed ($n = 46$) revealed a significant main effect of day, $F(1,42) = 5.88$, $p = .020$, $\eta_p^2 = .123$ (see Figure 5B), such that animals pressed more times on valued day ($M = 26.63$, $SD = 17.70$) compared to devalued day ($M = 22.50$, $SD = 16.65$). A Wilcoxon Signed Ranks Test, that included all animals collapsed across sex and order, also found a significant difference between valued and devalued days.

### Post-CTA Extinction

All animals met CTA criteria, with four animals (8.5%) needing all seven days of LiCl injections. The first post-CTA extinction trial active lever presses were analyzed using a between-subjects ANOVA comparing group, sex, and drug. See Table 1 for group sizes. There were no main effects of group, $F(1,39) = 0.74$, $p = .396$, sex, $F(1,39) = 2.24$, $p = .142$, or drug $F(1,39) = 0.21$, $p = .647$ (see Figure 6, Table 2). There were no significant interaction effects. Normality tests revealed that assumptions were violated with one outlier; however, removal of the outlier did not affect the results and so all data were included.

The second post-CTA extinction trial was analyzed in the same manner as the first extinction trial. No main effects of group, $F(1,39) = 0.06$, $p = .811$, sex, $F(1,39) = 0.05$, $p = .818$,

or drug, $F(1,39) = 0.12$, $p = .731$ (see Figure 7, Table 3). No significant interactions were found. Normality tests revealed assumptions were violated with one outlier, but removal of the outlier did not change the results and so all data were included.

A mixed-methods ANOVA was conducted on active lever presses to compare R121919 and vehicle treatments across both extinction trials. Four animals were excluded from analyses because they received vehicle treatment on both days ($n = 43$, 21 Devalued, 22 Valued). No interaction was found across day and treatment order, $F(1, 35) = 4.00$, $p = .053$, so data were collapsed across order. There were no main effects of group, $F(1, 39) = 0.47$, $p = .496$, sex, $F(1,39) = 0.95$, $p = .337$, or day, $F(1, 39) = 0.48$, $p = .494$ (Figure 8). There were also no significant interactions between day and group or day and sex.

# DISCUSSION

## General Discussion

This study looked at the effects of the CRF1 receptor antagonist R121919 on habit expression by measuring lever presses in an operant paradigm using sucrose as a reinforcer. Rats did not demonstrate meaningful devaluation in sensory-specific satiety extinction tests with no drug present and so were tested in a CTA paradigm. No group or drug differences were present during CTA extinction trials suggesting that while animals were not sensitive to devaluation, R121919 had no effect on lever pressing.

The major limitation is this study was the inability to demonstrate a successful devaluation. One possible reason for this is that rats may not have reach satiety in pre-feeding. If rats were not satiated during pre-feeding the value of sucrose as a reinforcer would have remained unchanged and lever pressing would not have been affected. Another possible reason for a failed devaluation is that rats might have already been exhibiting habitual behavior due to overtraining. Schoenberg et al. (2019) found that female Long Evans rats demonstrate habitual behavior after six VI30 sessions. Other studies have demonstrated habitual behavior using variable interval schedules in male Long Evans rats after 4 and 12 sessions (Lingawi & Balleine, 2012; Yin et al., 2004). During training, animals did not reach consistent responding and had variable response rates which led to an increase in the number of training session prior to devaluation testing. Animals were initially trained on 1.5% sucrose in operant chambers without prior exposure. Possible solutions would be to give animals access to sucrose in home cages prior to the start of training or start training with a higher concentration of sucrose which is then faded out to a lower final concentration in order to encourage responding (e.g. Windisch & Czachowski, 2018).

## Stress and Habit

Stress has been shown to shift animals from goal-directed responses to habitual behaviors (Dias-Ferreira et al., 2009). A disruption of top-down processes and an increase of activity in the dorsolateral striatum (DLS) most likely contribute to the shift to habit. In a reversal learning task, stressed animals switched to a DLS-dependent strategy (Snyder, Hill-Smith, Lucki, & Valentino,

2015) and this same pattern was shown in animals with intracerebroventricular injections of CRF (Hupalo, Bryce, et al., 2019). CRF could contribute to the disruption of higher order executive functions seen in stressed animals. For example, CRF in the nucleus accumbens made rats less sensitive to effort cost (Bryce & Floresco, 2019) and CRF activation in the mPFC impairs working memory (Hupalo, Martin, Green, Devilbiss, & Berridge, 2019). It is also known that high levels of CRF in the locus coeruleus switch animals to habit behavior (Hupalo, Bryce, et al., 2019). Stress might be influencing behaviors by decreasing executive function, and CRF appears to be acting in a similar manner. While there is a clear link between stress and CRF, and stress and habit, there is a lack on studies looking directly at the effects of CRF on habitual behaviors. It was hypothesized in this study that a CRF1 receptor antagonist would encourage goal-directed behavior in habitual animals because CRF and stress appear to promote habits. This could have uncovered a new possible mechanism for habit and an integration of stress, habit, and CRF literature.

The CRF1 receptor antagonist R121919 in this study had no effect on lever pressing in animals who were not sensitive to devaluation. The lack of a significant effect might have been because while excess CRF appears to bias animals towards habitual behavior, it may not influence habit in normal training paradigms. CRF can mimic the effects of stress (Bogdan et al., 2011). The effects of stress on CRF levels has been conflicting. While restraint stress appears to increase CRF levels in the amygdala (Kalin, Takahashi, & Chen, 1994), a footshock history alters CRF neurotransmission, but not density, in the locus coeruleus (Curtis, Pavcovich, Grigoriadis, & Valentino, 1995). Although CRF receptors have been reported in the DLS (De Souza et al., 1985) the effects of CRF in this brain region have been understudied. CRF may serve as a modulator between stress and habitual behaviors. Future studies should explore the relationship between stress and CRF in the DLS. In addition, it would be interesting to examine if stressed animals demonstrating habitual responding would revert to goal-directed behaviors after being given a CRF receptor antagonist. The effects of CRF on habit should be explored in the context of stress as CRF may serve as a mechanism of action for habit but only in stressed animals.

## Sex Differences

There are sex differences found in habitual behaviors, response to stress, and within the CRF system. Despite these known differences, the comparison of female and male animals remains an understudied field. In the current study there were no sex differences in active lever presses during training or any devaluation testing. Female rats did consume more sucrose in the chambers during training. Females rodents have been shown to be more prone to consume high-sugar palatable diets than males (Klump, Racine, Hildebrandt, & Sisk, 2013) and have a higher preference for glucose and saccharin (Valenstein, Kakolewski, & Cox, 1967). Compared to males, female rodents also generally consume more ethanol and a saccharine solution (Oberlin, Best, Matson, Henderson, & Grahame, 2011). There is evidence that sex differences may exist in habitual behaviors and could impact the effects of drugs of abuse. Differences in dopamine activity in the striatum may be linked to gonadal hormones (Barker & Taylor, 2019). Indeed, in female rodents estradiol and progesterone influence dopamine activity but have limited impact in males (Yoest, Quigley, & Becker, 2018). It has been shown that age of ethanol exposure and sex interact and shift females exposed in adolescence to habitual strategies in adulthood, but not males, and the same exposure in adulthood only has the opposite effect (Barker, Bryant, Osborne, & Chandler, 2017). In order to develop novel treatment options for AUD and substance use disorder it is important to know how drugs of abuse impact females and males differently.

I hypothesized that there would be sex differences in response to R121919 based on known differences in stress and CRF systems. Response to stress is different in females and males and this extends to CRF (Hodes & Epperson, 2019). For example, CRF that has been increased due to stress impairs attention in men but not women (Hodes & Epperson, 2019). CRF differences between females and males have been found in several brain regions including the dorsal raphe (Howerton et al., 2014), hippocampus (McAlinn et al., 2018), medial septum (Wiersielis et al., 2019), and locus coeruleus (Bangasser et al., 2010; Bangasser, Wiersielis, & Khantsis, 2016; Bangasser, Zhang, Garachh, Hanhauser, & Valentino, 2011). It has also been found that stress circuits activated by CRF differ between females and males (Salvatore et al., 2018). It is necessary to further explore possible sex differences in the CRF system in general, but also specifically as they relate to drugs of abuse and inflexible behaviors. Stress, CRF, and ethanol may all interact to help make the switch to habit, but there may be key differences in these mechanisms between females and males.

## Drugs of Abuse and Habit

CRF may have specific effects on habit only when ethanol is consumed or may perhaps rely on animals being dependent on alcohol. As previously discussed, in AUD the habit system is used more often than the goal-directed system (Vandaele & Janak, 2018) and alcohol and stress can increase glucocorticoids, stress hormones, which can lead to increased striatal activity (Blaine & Sinha, 2017). Ethanol is known to reduce orbitofrontal control, a structure that contributes to shifts in behavior, leading to an increase in habitual behaviors (Gremel & Costa, 2013; Renteria, Baltz, & Gremel, 2018). Drugs of abuse, including alcohol, disrupt dopamine in the striatum and lead to neuroadaptations that encourage habitual behavior (Schwabe, Dickinson, et al., 2011). Similar to the possible context of stress, CRF may have alcohol-specific effects in habit expression. Drugs of abuse can shape habitual behavior rapidly in what are sometimes referred to as pathological habits (Corbit, 2018). Future research should study the effects CRF might have on habits formed using drugs of abuse as a reinforcer. In a DID paradigm, a CRF1 antagonist decreased ethanol consumption and overall food intake and had no effect to a slight increase in sucrose consumption (Giardino & Ryabinin, 2013) suggesting that CRF could have reinforcer-specific effects. The effects of R121919 on habit expression should be further explored in animals responding for ethanol, dependent animals, or animals with a drinking history to find possible new mechanisms.

## Limitations and Future Directions

One limitation of the present study was that the rats took longer to train on an FR1 schedule than originally expected. Variability in lever pressing for sucrose and rats not achieving ordinary levels of responding led to unanticipated methodological changes. These changes included increasing of sucrose concentration, changing access to home cage drinking water, and increasing the amount of training days prior to a devaluation test all in an effort to have animals responding at a stable rate with minimal variability.

In order to draw meaningful conclusions from a sensory-specific satiety devaluation, animals must have at least one successful devaluation. This study found inconclusive results during the first and second extinction tests. When an outlier was removed on the second extinction test day a statistically significant difference in lever pressing was found between

valued and devalued days; however, with large variability across both days this effect may be minimal. It could be that the devaluation procedure was not successful or that rats may have been over-trained in the operant boxes prior to devaluation testing and therefore could have developed habitual behavior which would be insensitive to devaluation procedures.

Another limitation in the current study were small group sizes in the separate CTA extinction trials. Measuring across two time points within-subjects helped in increasing group size, particularly when collapsed across sex, but no effect of R121919 was seen in any of the groups. CRF1 receptor antagonists may not have an impact on habit expression but may influence habit formation. Future studies should examine the influence CRF may have on earlier stages of habit.

Future directions should also consider possible impacts of CRF2 receptors. A CRF2 receptor agonist have reduced binge-like ethanol drinking in both female and male mice and CRF2 receptor availability may impact CRF1 receptor antagonist effectiveness (Robinson et al., 2019). It is also known that other species such as prairie voles have increased CRF2 receptors, and that prairie voles share a similar monogamous nature and other hormone systems with humans (Potretzke, Robins, & Ryabinin, 2020). There is currently no CRF receptor radiotracer to detect CRF receptor levels or locations in the human brain. Using additional animal models such as prairie voles and focusing on CRF2 receptors could help with translational research to humans. CRF binding protein and the urocortins should also be included in future research as they may be impacting drinking behavior and have been understudied and may offer alternative explanations for mechanisms of action. CRF also interacts with many other neurotransmitters and hormones, such as orexin, which may be additional targets for understanding habitual behaviors (Kim & Martin-Fardon, 2020). Orexin is known to play a role in positive reward-seeking behaviors (Borgland et al., 2009; Harris, Wimmer, & Aston-Jones, 2005; Lawrence, Cowen, Yang, Chen, & Oldfield, 2006) in other areas of the corticostriatal system and may impact habit formation or expression.

Comparing differences in CRF receptors in the DLS, dorsomedial striatum (DMS), and the nucleus accumbens would also provide further insight into when CRF may be affecting habit. It has been shown that CRF influences dopamine release in the nucleus accumbens and increases cholinergic interneuron firing in both the nucleus accumbens and the dorsal striatum (Lemos et al., 2019). Examining CRF in the nucleus accumbens could provide insight into how CRF may

be acting on the DLS. Activity in the DLS and DMS during the switch from goal-directed to habitual behavior in early versus extended training is still not clear in how the two brain regions interact (Vandaele et al., 2019). Future research attempting to see how CRF might impact the switch between goal-directed and habitual behavior should investigate the interaction between these brain regions. CRF in alcohol use has often been studied in the context of withdrawal with a focus on the amygdala (Robinson et al., 2019; Valdez et al., 2002; Zorrilla, Logrip, & Koob, 2014). In habit, it is known that the basal lateral amygdala is first recruited but after extended training the central nucleus of the amygdala (CeA) maintains cocaine drug-seeking habits mediated by the DLS (Lipton, Gonzales, & Citri, 2019; Murray et al., 2015). The effects of CRF on the connection between the CeA and the DLS should be studied further.

## Conclusions

This study examined the CRF1 receptor antagonist R121919's effect on habit expression. R121919 had no effect on active lever presses in an extinction trial in animals that were not sensitive to devaluation after a CTA procedure. CRF could mediate the early stages of habit formation or may play a role in habit expression only when additional factors such as stress or alcohol are included. There were no sex differences in lever presses during operant training or in any of the extinction conditions; however, females did drink more sucrose in the chambers during training sessions than males. Future research should attempt to continue to examine the connection between CRF and habit possibly as it relates to the switch from goal-directed behaviors. New mechanisms of action could lead to new possible treatment options in conditions where an overactive habit system is present such as in AUD.

# TABLES

Table 1. Group Sizes in First and Second Post-CTA Extinction Trials.

| Group | Extinction One (*n*) | Extinction Two (*n*) |
|---|---|---|
| **R121919 Devalued** | 12 | 9 |
| **R21919 Valued** | 12 | 10 |
| **Vehicle Devalued** | 11 | 14 |
| **Vehicle Valued** | 12 | 14 |
| **Total** | 47 | 47 |

Table 2. Average Active Lever Presses During Post-CTA Extinction Test

| Drug | Female | | Male | | Total | |
|---|---|---|---|---|---|---|
| | **Devalued** | **Valued** | **Devalued** | **Valued** | **Devalued** | **Valued** |
| **R121919** | | | | | | |
| *M* | 30.17 | 40.83 | 52.50 | 62.83 | 41.33 | 51.83 |
| *SD* | 19.36 | 30.63 | 45.57 | 44.24 | 35.36 | 38.05 |
| **Vehicle** | | | | | | |
| *M* | 43.20 | 46.50 | 50.67 | 68.83 | 47.27 | 57.67 |
| *SD* | 29.35 | 37.84 | 32.22 | 74.10 | 29.64 | 57.30 |

*Note:* $N = 47$

Table 3. Average Active Lever Presses During Second Post-CTA Extinction Test

| **Drug** | **Female** | | **Male** | | **Total** | |
|---|---|---|---|---|---|---|
| | **Devalued** | **Valued** | **Devalued** | **Valued** | **Devalued** | **Valued** |
| **R121919** | | | | | | |
| *M* | 29.25 | 29.20 | 32.40 | 46.20 | 31.00 | 37.70 |
| *SD* | 13.25 | 16.12 | 21.84 | 35.84 | 17.52 | 27.69 |
| **Vehicle** | | | | | | |
| *M* | 53.57 | 27.43 | 34.14 | 36.43 | 43.86 | 31.93 |
| *SD* | 72.97 | 17.84 | 22.48 | 20.82 | 52.84 | 19.21 |

*Note:* N = 47

# FIGURES

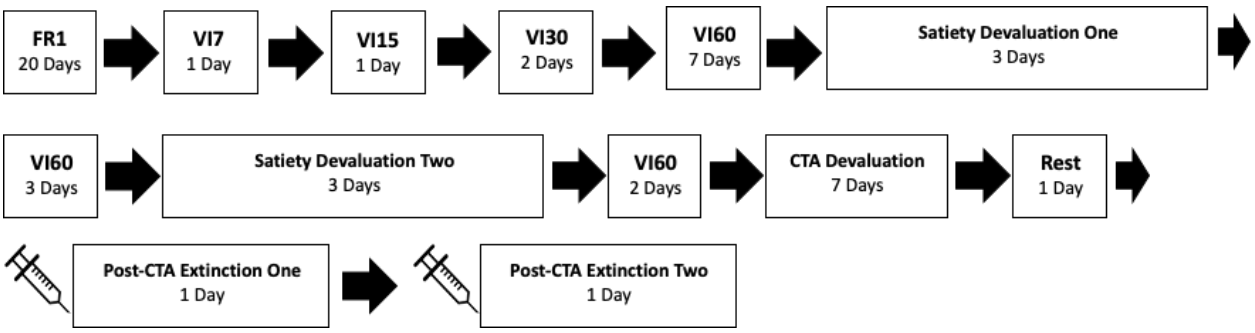


Figure 1. Timeline of Methodology

Flowchart of general timeline of operant training schedules and devaluation testing. Animals received an injection of either R121919 or vehicle prior to post-CTA extinction one and received the alternative treatment prior to post-CTA extinction two.

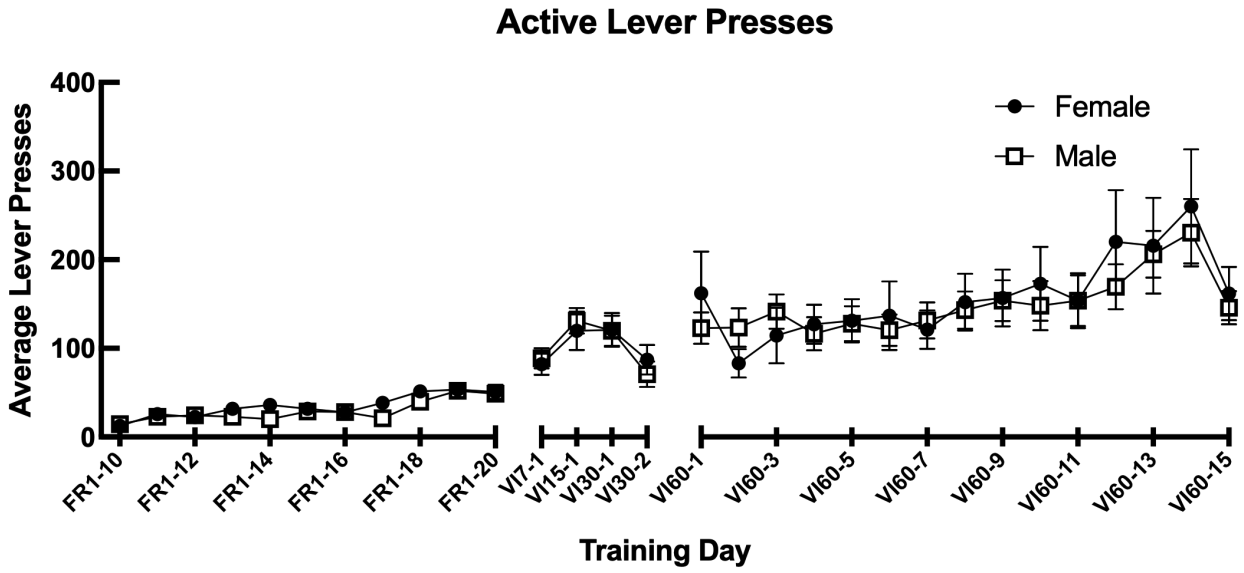


Figure 2. Average Active Lever Presses Across Training Days

Females and males increased active lever responding across training days. There are no sex differences. Mean and SEM for each sex shown across training days.

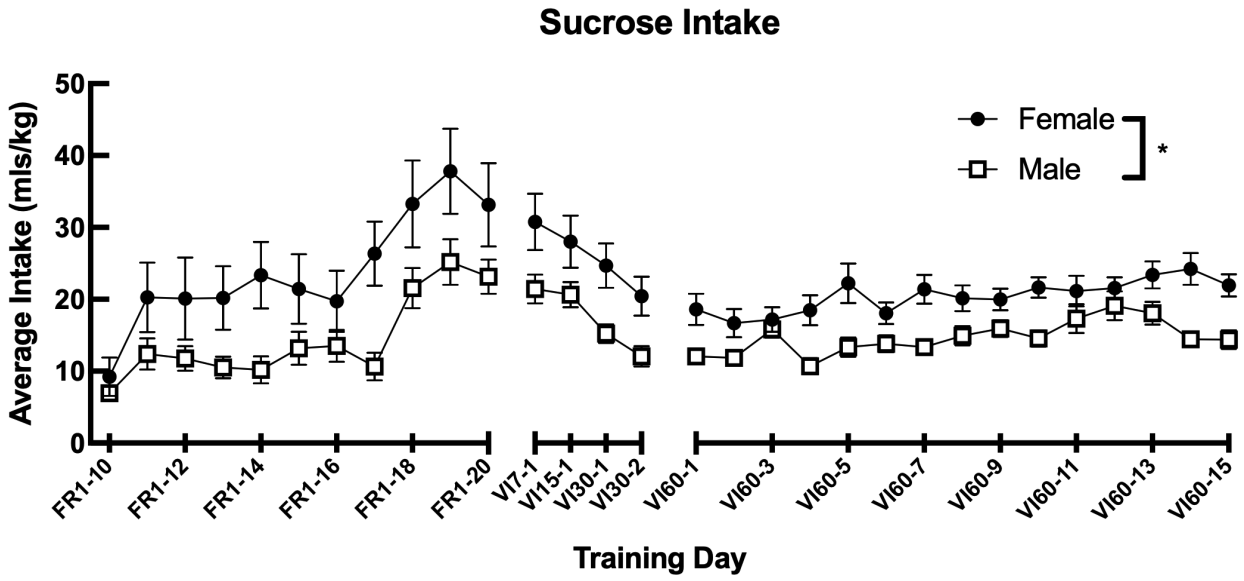


Figure 3. Average Sucrose Intake Across Training Days

Females consumed more sucrose than males across most days. Mean and SEM reported for each sex across training days. (*$p < .05$).

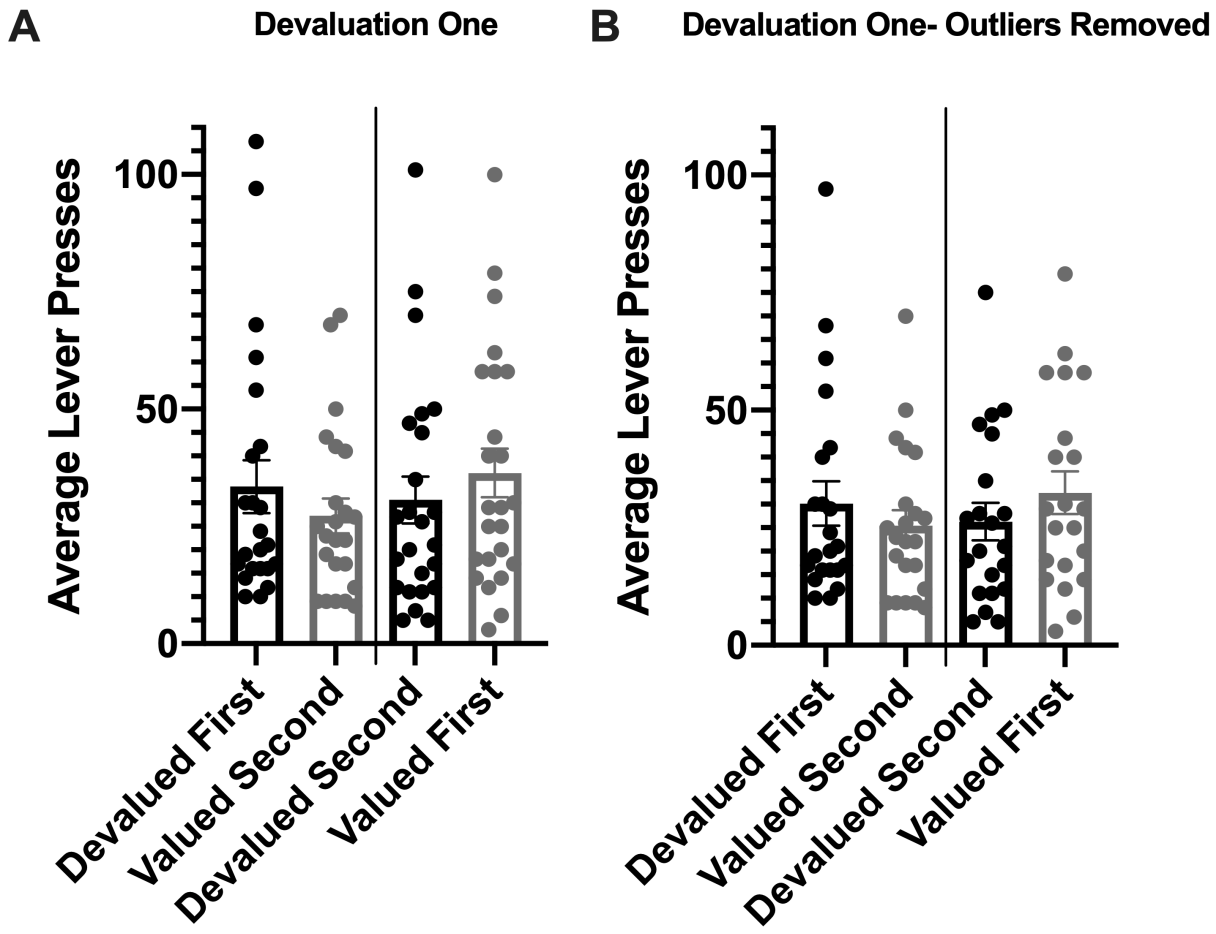


Figure 4. Average Lever Presses During Extinction Trials in First Devaluation Test

A significant effect of order was found so data were not collapsed across order. Animals who were devalued on day one (devalued first) pressed more on devalued day compared to valued (A). After outliers were removed it was revealed that animals that were valued on day one (valued first) pressed more on valued day than devalued (B). Mean and SEM reported for all groups.

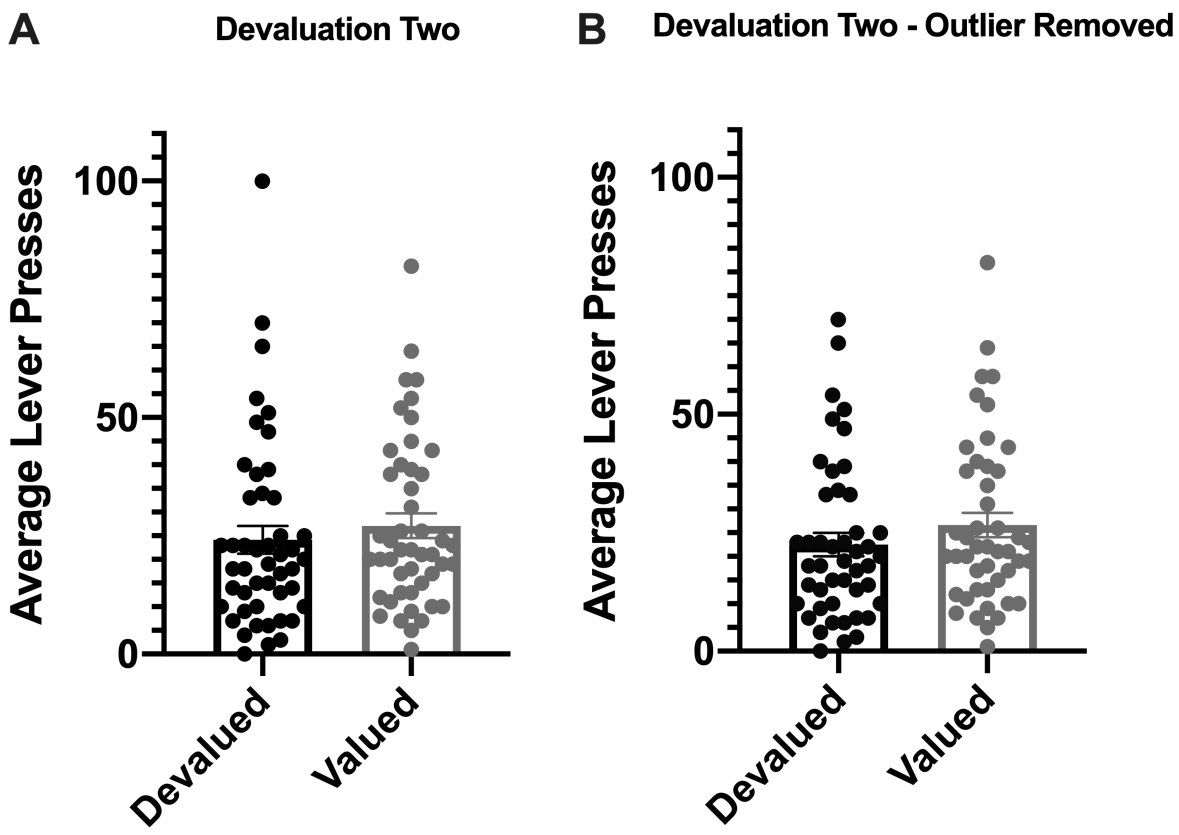


Figure 5. Average Lever Presses During Extinction Trials in Second Devaluation Test

Data were collapsed across order due to no significant effect. There was no difference in lever presses between devalued and valued days (A). After one outlier was removed a significant difference was found between devalued and valued days (B). Mean and SEM are shown.

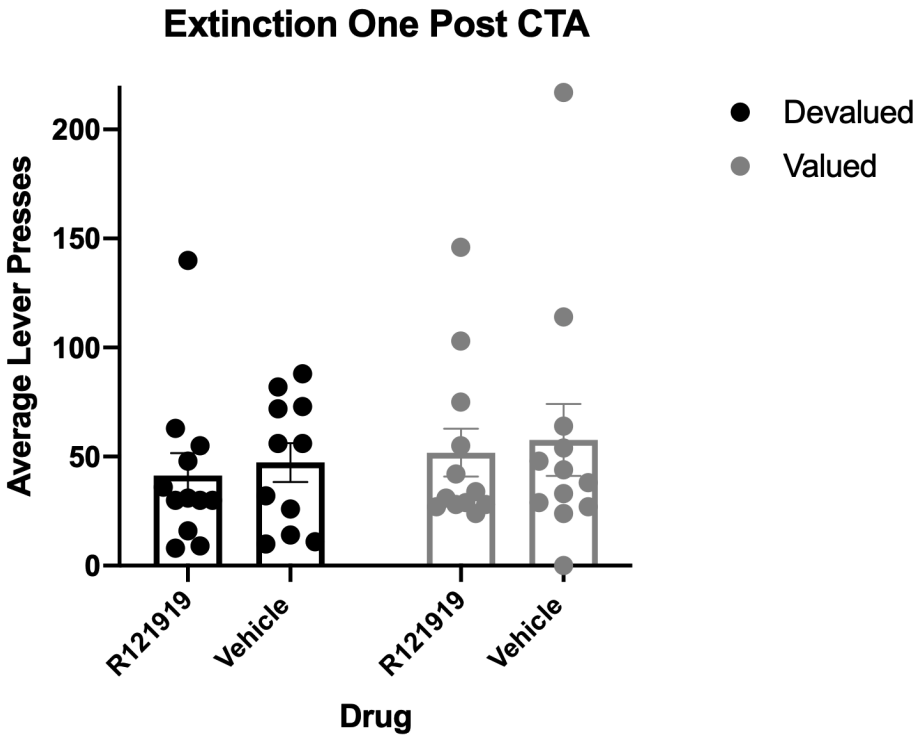


Figure 6. Active Lever Presses During Post-CTA Extinction One

No sex differences were present and so data were collapsed across sex. There were no differences in active lever presses between devalued or valued groups and no difference between R121919 and vehicle in either group. Mean and SEM are shown.

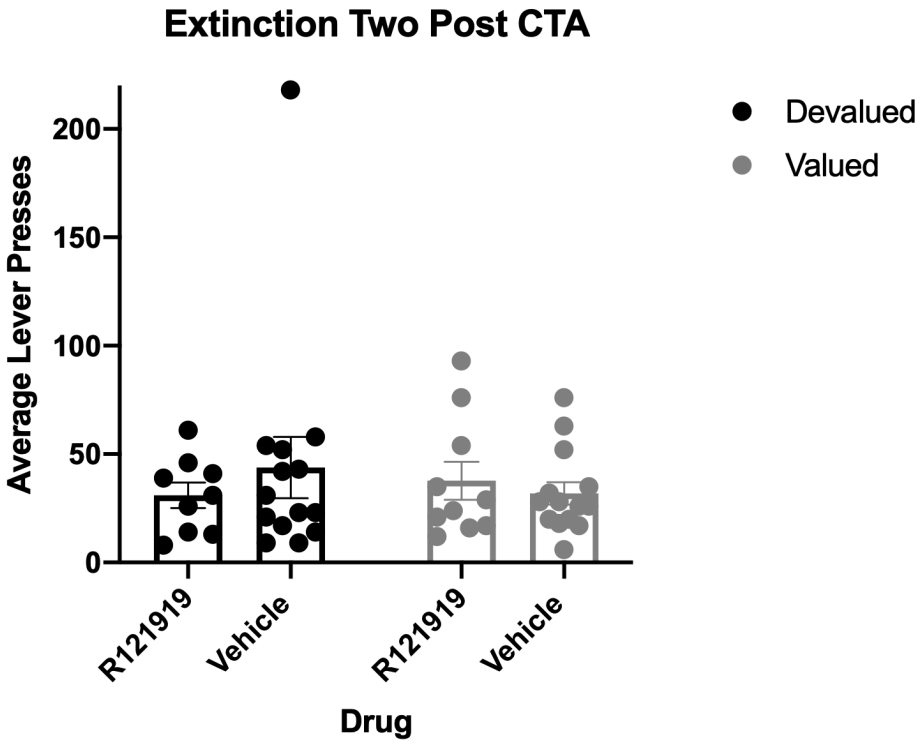


Figure 7. Active Lever Presses During Post-CTA Extinction Two

No sex differences were present and so data were collapsed across sex. There were no differences in active lever presses between devalued or valued groups and no difference between R121919 and vehicle in either group. Mean and SEM are shown.

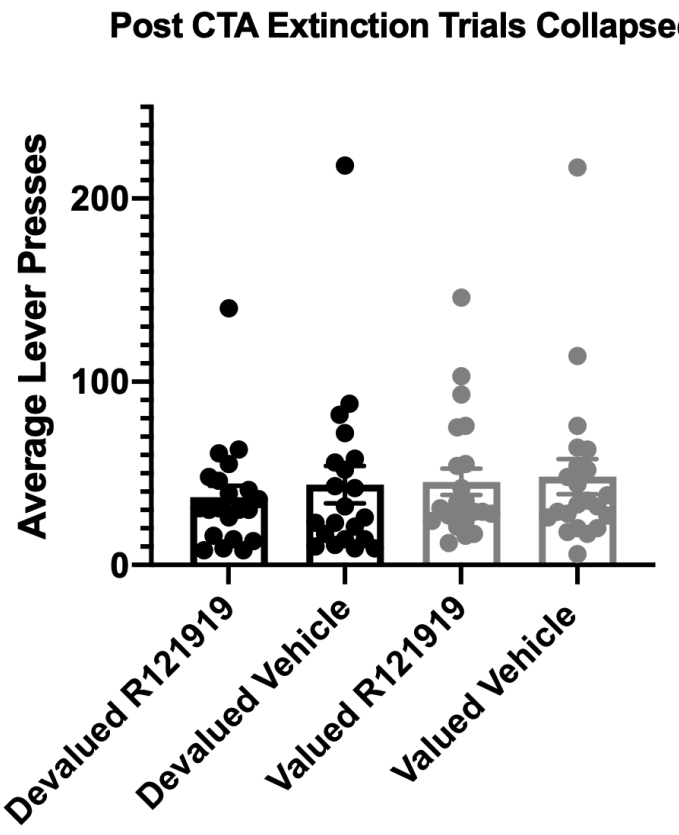


Figure 8. Active Lever Presses Collapsed Across Both Post-CTA Extinction Trials

Treatment was compared within-subjects across both extinction trials. There were no significant sex differences or order effects and so data shown are collapsed across both variables. There were no differences in active lever presses between R121919 and vehicle treatment days in either devalued or valued groups. Mean and SEM are shown for all groups.

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