# Extended access to oxycodone self-administration produces hyperalgesia and irritability-like behavior in male and female rats.

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**Abstract**

Over the last decade oxycodone has become one of the most widely abused drugs. The emergence of oxycodone dependence as a serious health crisis has prompted a major need for animal models of oxycodone dependence with face and predictive validity. Oxycodone use in humans is more prevalent in women (Administration, 2014) and leads to pronounced hyperalgesia and irritability. However, it is unclear if the current animal model of oxycodone self-administration recapitulates these characteristics. We assessed the face validity of an extended access oxycodone self-administration model in rats by examining escalation of oxycodone intake and behavioral symptoms of withdrawal including irritability like behavior and mechanical nociception in male and female rats. We found that male and female rats escalated oxycodone intake over the course of 14 self-administration sessions, however, female rats escalated took more drug than male rats once escalated. When we assessed irritability-like behavior we found no differences between baseline or withdrawal, however when tested immediately after a 12-h self-administration session rats showed a decreased number of aggressive responses and a increased number of defensive responses. When tested for mechanical threshold during withdrawal rats showed pronounced hyperalgesia that was only partially reversed by oxycodone self-administration. The results of the present study demonstrate the face validity of the extended access model of oxycodone self-administration by identifying sex differences in the escalation of oxycodone intake and demonstrating pronounced changes to pain and affective states.

**Keywords:** Oxycodone, von Frey, irritability, pain, hyperalgesia, self-administration, opioid

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## Introduction

Oxycodone is one of the most heavily abused prescription drugs in the world, and especially the United States (Manchikanti et al., 2010, Manchikanti and Singh, 2008, Administration, 2013). The prevalence of oxycodone use, and oxycodone related deaths have risen dramatically over the last decade bringing oxycodone to epidemic status (Kolodny et al., 2015, Compton et al., 2016). In order to combat the rising rate oxycodone addiction and determine viable treatment options it is critical to study oxycodone dependence using animal models that have strong translational validity.

In humans there have been significant sex differences identified in use of opioid pain relievers. Deaths from opioid overdose have increased much more rapidly for women than men over the last two decades (Prevention, 2017). This coincides with evidence that women abuse opioids at a higher rate than men (Administration, 2014). Compared to men, women may be more sensitive to pain (Riley et al., 1998) and are more likely to use opioids to cope with negative affect (McHugh et al., 2013), which may be a major factor in the increased rate of opioid abuse found in women.

Drug dependence and substance use disorder are thought to be driven by negative affect leading to escalated drug intake and withdrawal symptoms (Koob et al., 2004, Koob and Volkow, 2010). In humans opioid withdrawal can result in hyperalgesia and irritability in addition to other symptoms (Carcoba et al., 2011, Compton et al., 2003, Wesson and Ling, 2003, Gowing et al., 2017, Amato et al., 2013, Rieb et al., 2016). In order for animal models to have valid translational application they should mimic similar effects as seen in humans.

Although other opioids such as heroin and morphine have been heavily studied using animal models of self-administration (Wade et al., 2017, Schmeichel et al., 2015, Steidl et al.,

2015, Lucantonio et al., 2015, de Guglielmo et al., 2015) animal models of oxycodone self-administration are relatively understudied. Recent extended access studies have been shown to induce compulsive-like and escalated oxycodone intake, however behavioral withdrawal symptoms have not been explored (Zhang et al., 2014, Wade et al., 2015).

We sought to test the face validity of an extended access model of oxycodone self-administration by assessing at irritability-like behavior, which has been shown to be elevated during withdrawal from alcohol and nicotine (Sidhu et al., 2018, Somkuwar et al., 2017, Kimbrough et al., 2017, Xue et al., 2018, Kallupi et al., 2018), and hyperalgesia, which has been shown to be increased during withdrawal from alcohol, nicotine, and heroin (Kononoff et al., 2018, Edwards et al., 2012, Cohen et al., 2015, Park et al., 2015, de Guglielmo et al., 2017a, Kallupi et al., 2018), immediately after oxycodone use and during withdrawal. We hypothesized that the extended access animal model of oxycodone self-administration would result in escalated oxycodone intake and alterations to behavioral measures of withdrawal symptoms, indicating strong translational applications to humans.

## Materials and Methods

### *Animals*

Adult male and female Wistar rats (Charles River), aged to 60 days at the beginning of the experiments, were used. The rats were same sex group housed, two per cage, in a temperature-controlled (22°C) vivarium on a 12 h/12 h light/dark cycle (lights on at 8:00 PM) with *ad libitum* access to food and water. All of the procedures were conducted in strict adherence to the

National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by The Scripps Research Institute Institutional Animal Care and Use Committee.

*Experimental design*

Male and female rats were tested for baseline mechanical nociception and irritability-like behavior and then allowed to self-administer oxycodone for 14 days. On the second to last day of self-administration mechanical nociception was measured immediately after the 12-h self-administration session and 12-h into withdrawal. On the last day of self-administration irritability-like behavior was measured immediately after the 12-h self-administration session and 12-h into withdrawal **(Figure 1)**.

*Drugs*

Oxycodone (Sigma Aldrich, St. Louis, MO) was dissolved in 0.9% sodium chloride (Hospira, Lake Forest, IL, USA) and administered at a dose 150 μg/0.1 ml/kg.

*Intravenous catheterization*

The animals were anesthetized by inhalation of a mixture of isoflurane, and intravenous catheters were aseptically inserted in the right jugular vein using a modified version of a procedure that was described previously (Caine and Koob, 1993, de Guglielmo et al., 2013, de Guglielmo et al., 2017b). The vein was punctured with a 22-gauge needle, and the tubing was inserted and secured inside the vein by tying the vein with suture thread. The catheter assembly consisted of a 18 cm length of Micro-Renathane tubing (0.023 inch inner diameter, 0.037 inch outer diameter;

Braintree Scientific, Braintree, MA, USA) that was attached to a guide cannula (Plastics One, Roanoke, VA, USA). The guide cannula was bent at a near right angle, embedded in dental acrylic, and anchored with a mesh (2 Å thick, 2 cm square). The catheter exited through a small incision on the back, and the base was sealed with a small plastic cap and metal cover cap. This design helped to keep the catheter base sterile and protected. The catheters were flushed daily with heparinized saline (10 U/ml of heparin sodium; American Pharmaceutical Partners, Schaumburg, IL, USA) in 0.9% bacteriostatic sodium chloride (Hospira, Lake Forest, IL, USA) that contained 52.4 mg/0.2 ml of the antibiotic cefazolin.

*Oxycodone self-administration*

Each session was initiated by the extension of two retractable levers into the operant chamber (29 cm × 24 cm × 19.5 cm; Med Associates, St. Albans, VT, USA). Responses on the right active lever were reinforced on an FR1 schedule by intravenous (IV) oxycodone (150 μg/0.1 ml/infusion) administration that was infused over 6 s followed by a 20 s timeout (TO20 s) period that was signaled by the illumination of a cue light above the active lever for 14 days in 12-h daily sessions (5 sessions/week). Responses on the left inactive lever were recorded but had no scheduled consequences.

*Mechanical nociceptive von Frey testing*

Mechanical nociception, reflected by hindpaw withdrawal thresholds, was determined by an observer who was blind to the experimental condition using von Frey filaments, ranging from 8.511 to 281.838 g. The test was performed similarly to previous studies (Kononoff et al., 2018,

Kallupi et al., 2018). The test began after 10 min of habituation to the testing environment. A series of von Frey filaments was applied from below the wire mesh to the central region of the plantar surface of the left hindpaw in ascending order of force, beginning with the smallest filament (8.511 g). The filament was applied until buckling of the hair occurred, and the filament remained in place for 2 s. Rapid withdrawal of the hindpaw was considered a positive response. The stimulus was incrementally increased until a positive response was observed and then decreased until a negative response was observed to determine a pattern of responses to apply to the statistical methods previously described (Dixon, 1980). Once the threshold was determined for the left hindpaw, the same testing procedure was applied to the right hindpaw after 5 min. The 50% paw withdrawal threshold was determined by the formula $Xf + k\delta$, where $Xf$ is last von Frey filament applied, $k$ is the Dixon value that corresponded to the response pattern, and $\delta$ is the mean difference between stimuli. Paw withdrawal thresholds were determined for rats prior to self-administration (baseline), immediately after the second to last 12-h self-administration session, and 12 hours after the second to last self-administration session (12-h withdrawal).

*Irritability-like behavior*

To test irritability-like behavior during ethanol withdrawal and protracted abstinence, we used the bottle-brush test, based on the methods of Riittinen et al. (1986) and Lagerspetz and Portin (1968) and modified slightly for rats **(Kimbrough et al., 2017)**. Irritability-like behavior was tested prior to self-administration (baseline), immediately after the last 12-h self-administration sessions, and 12 hours after the last self-administration session (12-hour withdrawal). Irritability-like behavior was examined by measuring aggressive and defensive responses during the bottle-

brush test.

Irritability-like behavior sessions were conducted in a randomized order for each animal. Testing consisted of 10 trials per rat in plastic cages (10.5 in × 19 in × 8 in; Ancare, Bellmore, NY, USA) with fresh bedding. During each trial, the rat started at the back of the cage. A bottle-brush was rotated toward the animal's whiskers (from the front of the cage) by a treatment-naive experimenter. The brush was rotated around the whiskers of the rat for approximately 1 s. The brush was then rotated back to the front of the cage where it was allowed to hang vertically for approximately 2 s, during which behavioral responses were recorded. A 10-s intertrial interval was used. Three observers who were blind to treatment scored the behaviors in real time.

For each rat, separate sums of aggressive and defensive responses across all trials were determined for each observer. Aggressive and defensive response scores for each rat were then calculated by averaging the observers' sums. This was then used to calculate a group mean and SEM. The following were scored as aggressive responses: smelling the target, biting the target (during the initial phase of rotating the brush forward and back to the starting position), boxing the target, following the target, exploring the target (using paws or mouth to manipulate the brush without biting or boxing), mounting the target, and delayed biting (during the 2 s that the brush hung at the starting position). The following were scored as defensive responses: escaping from the target, digging, burying, defecation, jumping, climbing, vocalization, and grooming. Grooming and digging were additionally recorded during the 10-s intertrial intervals.

*Statistical analysis*

The results are expressed as mean ± SEM. Male and female oxycodone self-administration were analyzed separetly using a repeated-measures ANOVA with day of self-administration as the within-subjects factor and analyzed together using a repeated-measures ANOVA with sex as the between-subjects factor, and day of self-administration as the within-subjects factor. For each animal an average baseline value was calculated using the first 3 days of self-administration. This value was then used to compare individual animals' percent change from baseline for each day of self-administration. One sample t-tests versus a value of 100 were performed for each sex and each day to determine a significant percent change from baseline self-administration. Due to the sex differences found in daily self-administration we performed analysis of the average rewards received every 4-hour bin of the last 3 days of self-administration using separate within-subjects repeated measures ANOVAs for each sex. For irritability-like behavior and von Frey testing we first analyzed the data using a repeated measures ANOVA with sexes as the between-subjects factor and drug state as the within-subjects factor. We found no sex differences in irritability-like behavior or mechanical nociception, so we combined both sexes into a single group and analyzed each set of data using a repeated measures ANOVA with drug state as the within-subjects factor. For a secondary analysis of irritability-like behavior and mechanical nociception we represented the '12-h self-administration' drug state as a baseline value and determined percent change from this baseline at 12-h withdrawal. For mechanical nociception the percent change was calculated using the individual animals' thresholds at both time points. However, for the irritability-data some individual animals' values of 0 during the 12-h self-administration time point so we calculated the percent change of each individual animal at 12-h withdrawal from the group average value of the 12-h self-administration time point. One sample t-tests versus a value of 100 were performed for each behavior to determine if the change was

196 significant. The ANOVAs were followed by a Student Newman-Keuls (SNK) *post hoc* test when
197 appropriate. Differences were considered significant at $p < 0.05$. All of the data were analyzed
198 using Statistica 13 software (StatSoft, Palo Alto, USA).
199
200 **Results**
201 *Oxycodone self-administration*
202 We found that after 14 self-administration sessions both male and female rats significantly
203 escalated their oxycodone intake above the level of intake at day 1 **(Figure 2 A and B)**. When
204 male rats were analyzed separately, repeated measures ANOVA revealed a significant effect of
205 day $F_{13,117}= 9.97$, $p < 0.0005$. Post-hoc SNK showed that male rats had significantly greater
206 intake of oxycodone on days 6-14 (**Figure 2A**). When female rats were analyzed separately,
207 repeated measures ANOVA revealed a significant effect of day $F_{13,104}= 9.13$, $p < 0.0005$. Post-hoc
208 SNK showed that female rats had significantly greater intake of oxycodone on days 9-14 (**Figure**
209 **2B**). Male and female rats also showed significant percent increases in oxycodone self-
210 administration when compared to an average of the first 3 days of self-administration (**Figure**
211 **2D**).
212 When male and female rats were analyzed together to determine if sex differences
213 existed repeated measures ANOVA revealed a significant effect of day x sex interaction $F_{13,221}=$
214 2.13, $p < 0.05$. Post-hoc SNK showed males had significantly greater intake of oxycodone on
215 days 11 through 14 when compared to their own intake on day 1. Females had significantly
216 greater intake of oxycodone on days 8 through 14 when compared to their own intake on day 1.

Females were also found to have significantly greater intake of oxycodone than males on days 11 and 13 **(Figure 2C)**.

When we split the average intake of the last 3 sessions of self-administration into 4-hour bins (first 4 hours, middle 4 hours, and last 4 hours) for male rats the repeated measures ANOVA revealed a significant effect of hour $F_{2,18}= 6.26$, $p < 0.05$. Post-hoc SNK showed males had significantly greater intake of oxycodone during the first 4 hours (31.63±3.27) and middle 4 hours (29.27±3.72) when compared to the last 4 hours (24.40±2.76) of the session **(Figure 3A)**.

When we split the average intake of the last 3 sessions of self-administration into 4-hour bins (first 4 hours, middle 4 hours, and last 4 hours) for female rats the repeated measures ANOVA revealed a significant effect of hour $F_{2,16}= 4.37$, $p < 0.05$. Post-hoc SNK showed females had significantly greater intake of oxycodone during the middle 4 hours (47.07±7.94) when compared to the last 4 hours (33.48±3.24) of the session **(Figure 3B)**.

*Mechanical nociceptive von Frey response*

When rats were tested for paw withdrawal threshold using von Frey fibers we found no effect of sex and thus combined both sexes for the analyses. Repeated measures ANOVA for paw withdrawal threshold revealed a significant effect of drug state $F_{2,34}= 17.6$, $p < 0.0005$. Post-hoc SNK showed that the paw withdrawal threshold at baseline (33.62±4.19) was significantly higher than the threshold of after 12-h self-administration (24.50±3.39) and during 12-hour withdrawal (12.12±1.43). The paw withdrawal threshold after 12-h self-administration was also significantly higher than the threshold during 12-hour withdrawal **(Figure 4A)**. Rats also showed a significant percent decrease in paw withdrawal threshold from 12-h self-administration to 12-h withdrawal $t_{17}= 4.14$, $p < 0.005$ (**Figure 4B**).

*Irritability-like behavior*

When rats were tested for irritability-like behavior we found no effect of sex in either aggressive or defensive responses and thus combined both sexes for the analyses. Repeated measures ANOVA for aggressive responses revealed a significant effect of drug state $F_{2,36}= 7.12$, $p < 0.005$. Post-hoc SNK showed that the number of aggressive responses was significantly lower after 12-h self-adminsitration (2.79±0.69) compared to baseline (7.40±1.25) and during 12-hour withdrawal (8.04±1.13) **(Figure 4C)**. Rats also showed a significant percent increase in aggressive responses from 12-h self-administration to 12-h withdrawal $t_{18}= 4.64$, $p < 0.0005$ (**Figure 4D**).

Repeated measures ANOVA for defensive responses revealed a significant effect of drug state $F_{2,36}= 6.83$, $p < 0.005$. Post-hoc SNK showed that the number of defensive responses was significantly higher after 12-h self-administration (10.67±0.55) compared to baseline (7.60±0.95) and during 12-hour withdrawal (7.86±0.68) **(Figure 4C)**. Rats also showed a significant percent decrease in defensive responses from 12-h self-administration to 12-h withdrawal $t_{18}= 4.12$, $p < 0.005$ (**Figure 4D**). For an individual breakdown of each behavior see **Table 1**.

## Discussion

In the present study we assessed escalation of oxycodone self-administration and examined behavioral measures associated with withdrawal in male and female rats. We showed that an extended access model of oxycodone self-administration leads to escalation of intake in both male and female rats. However, female rats self-administered more drug one escalated,

indicating sex differences in oxycodone use. We also found that once rats had escalated oxycodone intake, irritability-like behavior was altered, immediately after the end of a 12-h self-administration session. Specifically, compared to baseline and 12-hour withdrawal time points rats showed increased defensive responses, and decreased aggressive responses. Finally, we found that hyperalgesia was altered by the state of drug usage (i.e. during withdrawal or after 12-h self-administration). After escalation of oxycodone intake, immediately after the end of a 12-h self-administration session, rats showed a small increase in pain sensitivity compared to baseline sensitivity. We further found that 12-hours into withdrawal, from oxycodone self-administration, rats had a pronounced hyperalgesia sensitivity compared to both baseline and end of a 12-h self-administration time points.

These results show that the extended access rat model of oxycodone self-administration leads to reliable escalation of drug use and alterations to behavioral measures of withdrawal. Interestingly, although both male and female rats show a slight reduction in intake during the last 4 hours of self-administration, overall the intake remains relatively constant, suggesting that a sufficient amount of drug should be in the system during our testing immediately after the sessions. Furthermore, the fact that the rats self-administer heavily throughout the 12-hour session indicates the benefit of the extended duration session in modeling oxycodone dependence.

We found changes to irritability-like behaviors at the end of a 12-h oxycodone self-administration when compared to baseline and 12-hour withdrawal, suggesting oxycodone itself may be mediating the changes to aggressive and defensive behavior. However, our baseline measures for pain and irritability were taken prior to the initiation of any drug use and although we see changes in pain during 12-hour withdrawal compared to baseline it is possible that the

more relevant comparison for pain and irritability is 12-h withdrawal versus after 12-h self-administration, when drug is still in the system, since at this stage of the experiment the animals alternate between these two conditions, 12-hour withdrawal and 12-hour access to oxycodone. If we consider after 12-h self-administration as a new 'baseline' value after dependence has been induced., then 12-h withdrawal from this new baseline is associated with an increase in aggressive behavior and decrease in defensive behavior. Similarly, the hyperalgesia data demonstrate that after escalation of oxycodone self-administration rats exhibit a new baseline state of hyperalgesia that is present after 12-h self-administration and during 12-h withdrawal.

We found that female rats self-administered more oxycodone, which mirrors human findings that abuse of, and craving for, prescription pain medications and opioids is more frequent in women (Back et al., 2011, Administration, 2014). Opioid related deaths have risen significantly more in women than men over the past 10 years (Choo et al., 2014). Human dependence on opioids has been found to be associated with increased aggression, presumably due to bouts of withdrawal (McKernan et al., 2015, Moore et al., 2011). Our current animal model data matches the human findings, as we see increased aggressive responses during 12-h withdrawal when compared to after drug use (12-h self-administration). Additionally, the increases we find in hyperalgesia match human phenotypes. Similar to our data, in humans withdrawal from opioids leads to an increased level of pain (Gowing et al., 2017, Rieb et al., 2016, Carcoba et al., 2011, Compton et al., 2003). Interestingly, immediately after 12-h oxycodone self-administration we found the rats had a slightly increased level of pain. Although at first this may seem odd, as opioid use is often for pain relief purposes (Olesen et al., 2010), similar effects have been seen in mice and humans. In a mouse model of pain, a 10-day course of morphine was found to prolong pain duration (Grace et al., 2016). Human studies have shown

that opioid use can result in increased pain, which has been termed opioid-induced hyperalgesia (Angst and Clark, 2006, Chu et al., 2008). Additionally, the current findings match models of self-administration in other opioids, such as heroin, where hyperalgesia is found during withdrawal from dependence (Edwards et al., 2012, Park et al., 2015).

Taken together, our current findings suggest that the rat long access model of oxycodone self-administration has strong face validity for studying opioid use disorders. We showed sex differences in intake and found alterations to irritability-like behavior and hyperalgesia similar to humans. Overall these findings demonstrate that the extended access model to oxycodone self-administration is a valid model for future studies seeking to identify novel therapeutic approaches for opioid use disorder and identify the neurobiological basis of oxycodone dependence and addiction.

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470 **Conflicts of interest**

471 The authors declare no conflicts of interest.

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## Figure Legends

**Figure 1.** Experimental design. Male and female rats were tested for baseline mechanical nociception and irritability-like behavior and then allowed to self-administer oxycodone for 14 days. On the second to last day of self-administration mechanical nociception was measured immediately after the self-administration session and 12-h into withdrawal. On the last day of self-administration irritability-like behavior was measured immediately after the self-administration session and 12-h into withdrawal.

**Figure 2.** Escalation of oxycodone self-administration. **A.** Oxycodone self-administration in male rats. Males showed a significant increase in active lever presses (black circles) compared to day 1 on days 6-14, but no change in inactive lever presses (white circles). **B.** Oxycodone self-administration in female rats. Females showed a significant increase in active lever presses (black circles) compared to day 1 on days 9-14, but no change in inactive lever presses (white circles). **C.** Active lever presses in male and female rats over the course of oxycodone self-administration. Female rats (white circles) showed significantly increased oxycodone lever presses compared to day 1 on days 8-14. Male rats (black circles showed significantly increased oxycodone lever presses compared to day 1 on days 11-14. Female rats also showed significantly higher oxycodone lever presses compared to male rats on days 11 and 13. **D.** Percent change in oxycodone intake from first 3 days average baseline intake in male and female rats. Male rats (black circles) showed a significant percent increase in oxycodone intake from baseline on days 5, 6, and 8-14. Female rats (white circles) showed a significant percent increase in oxycodone

intake from baseline on days 8, 9, 10, and 12-14. *p < 0.05 oxycodone lever presses on day versus intake on day 1/baseline. #p < 0.05 male vs. female oxycodone lever presses on the specific day.

**Figure 3.** Average 4-hour bins during the last 3 days of self-administration. **A.** Male self-administration averages for the last 3 days in 4-hour bins. Male rats self-administered significantly less oxycodone in last 4 hours of self-administration compared to the first 4 hours and middle 4 hours. **B.** female self-administration averages for the last 3 days in 4-hour bins. Female rats self-administered significantly less oxycodone in last 4 hours of self-administration compared to the middle 4 hours. *p < 0.05 oxycodone rewards in the last 4 hours versus the first 4 hours 1. #p < 0.05 oxycodone rewards in the last 4 hours versus the middle 4 hours.

**Figure 4. A.** Paw withdrawal threshold measured by von Frey filaments. Rats showed a significantly lower paw withdrawal threshold 12 hours into withdrawal (black bar) than baseline (white bar) and at the end of a self-administration session (12-h self-administration; hashed bar). Rats also showed a significantly lower paw withdrawal threshold at the end of a self-administration session compared to baseline. **B.** Rats showed a significant percent decrease in paw withdrawal threshold from the end of self-administration (black bar) to 12-h withdrawal (white bar). **C.** Irritability-like behavior. Rats showed a significantly higher number of defensive responses at the end of a self-administration session (12-h self-administration; hashed bars) compared to baseline (white bars) and during 12-hour withdrawal (black bars). Rats showed a significantly lower number of aggressive responses at the end of a self-administration session

compared to baseline and during 12-hour withdrawal. **D.** Rats showed a significant percent decrease in defensive responses from the end of self-administration (black bars) to 12-h withdrawal (white bars). Rats showed a significant percent increase in aggressive responses from the end of self-administration to 12-h withdrawal. *p < 0.05 vs. baseline. #p < 0.05 vs. 12-hour withdrawal. *p < 0.05 vs. baseline. #p < 0.05 vs. 12-hour withdrawal.

**Table 1.** Individual irritability-like behaviors. Within-subjects repeated measures ANOVAs were performed for each behavior. The F/p-values are shown if significant and marked as *n.s.* for non-significant effects, and *n.p.* for tests not performed due to data with no variance in one or more groups (e.g. all values of 0 for a behavior in one group). *Post-hoc* SNK were performed if the within-subjects repeated measures ANOVA was significant for an effect of drug state. If the ANOVA was not significant the *post-hoc* comparison was marked *n.a.*, if the *post-hoc* comparison was not significant the value was marked as *n.s.*

| | | Baseline (BSL) | | 12-h Self-administration (SA) | | 12-hour Withdrawal (WD) | |
|---|---|---|---|---|---|---|---|
| **Behavior** | **Repeated measures ANOVA F/p-values** | **AVG±SEM** | ***Post-hoc* SNK p** | **AVG±SEM** | ***Post-hoc* SNK p** | **AVG±SEM** | ***Post-hoc* SNK p** |
| Escape | $F_{2,36}$= 23.09, p<0.0005 | 4.35±0.50 | p<0.05 vs. SA<br>p<0.0005 vs. WD | 5.40±.0.33 | p<0.05 vs. BSL<br>p<0.0005 vs. WD | 2.28±.0.27 | p<0.0005 vs. BSL<br>p<0.0005 vs. WD |
| Digging | *n.p* | 0.05±.0.05 | *n.a.* | 0.02±.0.02 | *n.a.* | 0.00±.0.00 | *n.a.* |
| Jumping | $F_{2,36}$= 3.82, p<0.05 | 0.81±.0.34 | p<0.05 vs. WD | 0.40±.0.21 | *n.s.* | 0.05±.0.05 | p<0.05 vs. BSL |
| Climbing | $F_{2,36}$= 18.75, p<0.0005 | 1.88±.0.27 | p<0.0005 vs. SA<br>p<0.0005 vs. WD | 4.39±.0.41 | p<0.0005 vs. BSL | 3.84±.0.42 | p<0.0005 vs. BSL |
| Defecation | *n.p.* | 0.11±.0.07 | *n.a.* | 0.00±.0.00 | *n.a.* | 0.12±.0.12 | *n.a.* |
| Vocalization | $F_{2,36}$= 3.34, p<0.05 | 0.30±.0.23 | p<0.05 vs. WD | 0.19±.0.12 | *n.s.* | 1.09±.0.45 | p<0.05 vs. WD |
| Grooming | *n.s.* | 0.11±.0.11 | *n.a.* | 0.26±.0.16 | *n.a.* | 0.47±.0.16 | *n.a.* |
| **Total Defensive** | $F_{2,36}$= 6.83, p<0.005 | **7.60±.0.95** | p< 0.05 vs. SA | **10.67±.0.55** | p< 0.05 vs. BSL<br>p< 0.005 vs. WD | **7.86±.0.68** | p< 0.005 vs. SA |
| Smelling | $F_{2,36}$= 3.50, p<0.05 | 1.53±.0.39 | *n.s.* | 0.63±.0.19 | p<0.05 vs. WD | 2.00±.0.43 | p<0.05 vs. SA |
| Biting | *n.s.* | 0.02±.0.02 | *n.a.* | 0.04±.0.04 | *n.a.* | 0.14±.0.06 | *n.a.* |
| Delayed Biting | *n.s.* | 0.05±.0.04 | *n.a.* | 0.02±.0.02 | *n.a.* | 0.18±.0.09 | *n.a.* |
| Boxing | $F_{2,36}$= 6.57, p<0.005 | 2.02±.0.39 | p<0.05 vs. SA | 0.54±.0.15 | p<0.05 vs. BSL | 2.19±.0.52 | p<0.05 vs. SA |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | p<0.05 vs. WD | | |
| Following | $F_{2,36}$= 3.33, p<0.05 | 2.40±.0.55 | *n.s.* | 0.86±.0.28 | p<0.05 vs. WD | 2.32±.0.48 | p<0.05 vs. SA |
| Exploration | *n.s.* | 1.39±.0.24 | *n.a.* | 0.70±.0.21 | *n.a.* | 1.21±.0.22 | *n.a.* |
| **Total Aggressive** | $F_{2,36}$= 7.12, p< 0.005 | **7.40±.1.25** | p< 0.005 vs. SA | **2.79±.0.69** | p< 0.005 vs. BSL<br>p< 0.005 vs. WD | **8.04±.1.13** | p< 0.005 vs. SA |

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