Intermittent abstinence potentiates oxycodone self-administration under extended access conditions and alters brain reward sensitivity

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Keywords: Oxycodone, Self-Administration, Escalation

ABSTRACT

Prescription opioid addiction is a significant health problem characterized by compulsive drug seeking, withdrawal and chronic relapse. This study investigated the neurobiological consequences of escalation of prescription opioid use using an oxycodone intravenous self-administration (IVSA) model in rats. Male Wistar rats were trained oxycodone self-administration (0.15 mg/kg/infusion, i.v.) in 1h or 11-12h access sessions. Rats given 12 h Long Access (LgA) to oxycodone for 5 sessions/week escalated their responding more than rats given 1h oxycodone Short Access (ShA). Interestingly, a significant increase in oxycodone intake was observed after 60 h weekend discontinuations from drug access. A separate group of rats was trained in the intracranial self-stimulation (ICSS) reward procedure and then intravenous oxycodone self-administration in LgA sessions. Pre-IVSA brain reward thresholds increased with sequential daily LgA IVSA sessions, consistent with a growing negative affective state consequent to successive daily abstinence cycles. A 1 h IVSA interval was sufficient to normalize these elevated reward thresholds. Interestingly, the brain reward thresholds normalized to baseline across 60 h weekend abstinences. This latter finding is consistent with a normalization of brain reward status suggesting the associated increased IVSA of drug was not driven entirely by a persisting negative affective state. Escalated oxycodone taking when abstinence periods vary may be driven by a complex mix of negative and positive reinforcing effects of the drug.

Introduction

Nonmedical opioid abuse is a significant global problem, with an estimated 33 million users of opiates and prescription opioids worldwide (UNODC, 2016). Approximately 2 million people in the US have a prescription opioid related abuse disorder (CBHSQ, 2015), which may increase the likelihood of later illicit opioid use (Muhuri, 2013), and prescription opioid related overdose deaths have drastically increased over the last two decades (CDC, 2016). Despite the growing impact of prescription opioids on public health, relatively few pre-clinical studies have investigated self-administration of oxycodone, one of the most commonly prescribed medications (OxyContin® or as part of Percocet®). The available pre-clinical studies have shown that oxycodone self-administration causes behavioral changes (Zhang *et al*, 2016), and sometimes physical dependence and withdrawal (Enga *et al*, 2016) in mice. Oxycodone self-administration is similar between male and female rats during early stages of training (Mavrikaki *et al*, 2017), and male rats trained to self-administer oxycodone under extended access conditions (12h) exhibited a progressive escalation of drug intake (Wade *et al*, 2015) similar to heroin escalation under similar 12h access conditions (Schlosburg *et al*, 2013; Vendruscolo *et al*, 2011). Mice also exhibit escalation of oxycodone self-administration under 4h extended access conditions (Zhang *et al*, 2014).

The negative reinforcement hypothesis that has been advanced to explain escalating drug intravenous self-administration (IVSA) under extended-access conditions (George *et al*, 2014; Koob, 2015) holds that the dysphoric or negative affective state that is experienced during the daily withdrawal of drug access grows increasingly severe with sequential sessions. Intracranial self-stimulation (ICSS) reward data have been crucial to arguments that the escalation phenotype is not due to primary pharmacodynamic tolerance but instead reflects dysregulation of common reward and affective neuronal circuitry. It has been shown that brain reward thresholds progressively increase with the self-administration of heroin in 23h but not 1h daily sessions (Kenny *et al*, 2006a). Similarly, ICSS reward thresholds were increased compared to baseline in rats trained to self-administer methamphetamine in 6 h but not 1 h IVSA sessions (Jang *et al*, 2013) or cocaine under 6h access (Ahmed *et al*, 2002), with evidence of recovery back to baseline that persisted during at least 8 days post-escalation in the latter study. Correspondingly, the somatic signs of withdrawal from heroin increase progressively from 12 to 48h in adult rats (Doherty and Frantz, 2013). Further, studies have also shown that withdrawal from ethanol (Schulteis *et al*, 1995), nicotine (Epping-Jordan *et al*, 1998) or amphetamine (Lin *et al*, 1999) results in elevated reward thresholds in rats. These observations suggest that elevations in reward thresholds may index the anhedonic symptoms associated with withdrawal from drugs of abuse (Der-Avakian and Markou, 2012). Together these findings suggest that motivation

for drug-seeking is closely related to brain reward systems reflected in ICSS thresholds and together are predictive measures of abuse-related drug effects. A study by Wiebelhaus and colleagues showed that acute and repeated injections of oxycodone alter ICSS responding (Wiebelhaus *et al*, 2016); however the effects of oxycodone self-administration on ICSS brain reward thresholds have not been investigated.

This study was designed to determine if the escalation of oxycontin self-administration that is associated with extended (11-12h) access (Wade *et al*, 2015) is accompanied by decreased brain reward function using the ICSS procedure. The goal was to test the hypothesis that continued cycles of extended intoxication, followed by daily abstinence intervals, facilitates an increase in drug taking driven by a progressively negative affective state.

Materials and Methods

Animals. Male Wistar rats (N=37; Charles River, Raleigh, NC) were housed in a humidity and temperature-controlled (23±1°C) vivarium on 12:12h light:dark cycles. Animals entered the laboratory at 11-14 weeks of age and weighed an average of 410.0±5.56 g at the start of the self-administration study. Animals had *ad libitum* access to food and water in their home cages and were housed in pairs throughout the study. All procedures were conducted in the animals' scotophase, under protocols approved by the Institutional Care and Use Committees of the Scripps Research Institute and consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Garber *et al*, 2011).

Intravenous Catheterization. Rats were anesthetized with an isoflurane/oxygen vapor mixture (isoflurane 5% induction, 1-3% maintenance) and prepared with chronic indwelling intravenous catheters as described previously (Aarde *et al*, 2013; Nguyen *et al*, 2016b; Nguyen *et al*, 2017a). Briefly, the intravenous catheters consisted of a 14.5-cm length of polyurethane based tubing (Micro-Renathane®, Braintree Scientific, Inc, Braintree, MA) fitted to a guide cannula (Plastics One, Roanoke, VA) curved at an angle and encased in dental cement anchored to an ~3 cm circle of durable mesh. Catheter tubing was passed subcutaneously from the animal's back to the right jugular vein. Catheter tubing was inserted into the vein and tied gently with suture thread. A liquid tissue adhesive was used to close the incisions (3M™ Vetbond™ Tissue Adhesive: 1469SB, 3M, St. Paul, MN). A minimum of 4 days was allowed for surgical recovery prior to starting an experiment. For the first three days of the recovery period, an antibiotic (cefazolin) and an analgesic (flunixin) were administered daily. During testing and training, intravenous

catheters were flushed with ~0.2-0.3 ml heparinized (166.7 USP/ml) saline before sessions and ~0.2-0.3 ml heparinized saline containing cefazolin (100 mg/mL) after sessions.

Catheter patency was assessed once a week after the last session of the week, via administration through the catheter of ~0.2 ml (10 mg/ml) of the ultra-short-acting barbiturate anesthetic Brevital sodium (1% methohexital sodium; Eli Lilly, Indianapolis, IN). Animals with patent catheters exhibit prominent signs of anesthesia (pronounced loss of muscle tone) within 3 sec after infusion. Animals that failed to display these signs were considered to have faulty catheters, and if catheter patency failure was detected, data that were collected after the previous passing of this test were excluded from analysis.

Drugs. Oxycodone HCl was obtained from Sigma-Aldrich (St. Louis, MO). All doses are expressed as the salt and were dissolved in physiological saline (0.9%).

Self-Administration Procedure. *Acquisition*: Drug self-administration was conducted in operant boxes (Med Associates, Inc., Fairfax, VT) located inside sound-attenuating chambers located in an experimental room (ambient temperature 22±1 °C; illuminated by red light) outside of the housing vivarium. To begin a session, the catheter fittings on the animals' backs were connected to polyethylene tubing contained inside a protective spring suspended into the operant chamber from a liquid swivel attached to a balance arm. Each operant session started with the extension of two retractable levers into the chamber. Following each completion of the response requirement (response ratio), a white stimulus light (located above the reinforced lever) signaled delivery of the reinforcer and remained on during a 20-sec post-infusion timeout, during which responses were recorded but had no scheduled consequences. Drug infusions were delivered via syringe pump. The training dose (0.15 mg/kg/infusion; ~0.1 ml/infusion) was selected from prior self-administration studies (Wade *et al*, 2015). The session duration for the Short Access (ShA) group was 1 h and the Long Access (LgA) training sessions were 12h (or 11h for ICSS-trained rats) in duration. Self-administration sessions were scheduled during weekdays with drug abstinence on the weekend.

Intravenous Self-Administration of Oxycodone Under Short and Long Access Conditions. Rats were randomly assigned to 12 h LgA (N=12) and 1 h ShA (N=12) groups. Two rats in the LgA group that failed to average more than 24 infusions (defined by the highest responder in a Saline LgA group, see (Nguyen *et al*,

2017b)) over the final 5 sessions of acquisition were excluded from the acquisition and PR self-administration data as non-escalators. Three rats in the LgA group failed patency testing during the acquisition interval and were excluded. One additional rat from the LgA group was euthanized during abstinence due to illness. Rats were returned to their home cages for an extended 30-day abstinence period and then returned to 12h sessions to test for re-engagement of drug seeking (re-escalation) following detoxification.

Progressive-Ratio (PR) Dose-Response Testing: Following acquisition rats were subjected to randomized dose-substitution conditions under a Progressive Ratio (PR) schedule of reinforcement wherein different per-infusion doses of oxycodone (0, 0.06, 0.15, 0.3 mg/kg/inf) were presented in a balanced order on sequential sessions lasting up to 3h. For the PR task, the sequence of response ratios started with one response then progressed thru ratios determined by the following equation (rounded to the nearest integer): response ratio=5e^(injection number x j) – 5 (Richardson and Roberts, 1996). The value of 'j' was 0.2 and was chosen so as to observe a 'breakpoint' within ~3h. The last ratio completed before the end of the session (1h after the last response up to a maximum 30h sessions) was operationally defined as the breakpoint. The dose order was balanced by Latin Square design (i.e. the 4 total conditions were randomized).

Intracranial Self-Stimulation (ICSS) Reward During Intravenous Self-Administration Of Oxycodone. For these studies, a separate cohort of rats (N=18) were anesthetized and prepared with unilateral electrodes aimed at the medial forebrain bundle (coordinates: AP -0.5mm, ML ±1.7mm, DVskull -9.5mm). Animals were trained in a procedure adapted from the discrete-trial current-threshold procedure (Kenny and Markou, 2006b; Kornetsky and Esposito, 1979; Markou and Koob, 1992; Nguyen *et al*, 2016a). Trials begin with a noncontingent stimulation (sinusoidal electrical stimuli of 250 ms duration and 60Hz), followed by a variable post-stimulation interval (7.5 s) during which delivery of a second stimulus was contingent upon responding with a ¼ turn of a wheel manipulandum. Each electrical stimulation (reinforcer) had a train duration of 500 ms during which 0.1 ms cathodal pulses were delivered at 50-100 Hz, with current-intensity thresholds within 50-200 μA. Current was varied in a series of steps (±5 μA per step, 3 trials per step). In each testing session, four alternating descending-ascending series were presented. The threshold for each series was defined as the midpoint between two consecutive current intensities that yielded 'positive scores' (animals responded for at least two of the three trials) and two consecutive current intensities that yielded 'negative scores' (animals did not respond for two or more of the three trials). The

overall threshold of the session was defined as the mean of the thresholds for the four individual series. Each testing session was ~30 min in duration. Rats were trained once daily until stable reward threshold were established (≤10% variation in thresholds for three consecutive days) between 7 and 10 days. Following ICSS training, rats were implanted with intravenous catheters (see above). After a 7 day recovery, the rats were tested in the ICSS experiments for 5 days without drug exposure to determine baseline reward thresholds (average of the 5 days). Starting the following week ICSS sessions were run ~1hr prior to each oxycodone self-administration session.

Self-administration in ICSS Trained Rats. Rats completed daily (M-F) self-administration sessions after ICSS sessions for three sequential weeks, with the self-administration session omitted on the Th of Week 3. Six rats were randomly assigned to ShA (1 h) and 11 rats to LgA (11 h) since the primary goal was to assess LgA IVSA. One rat was excluded from the study due to opioid-induced self-injury during acquisition. The effect of non-contingent oxycodone (see below) was assessed during weeks 4-5 and again in Week 10. Week 6 constituted an uninterrupted training week of ICSS followed by IVSA sessions. Self-administration sessions were omitted during Week 7. In Week 8, rats received a second ICSS evaluation after one hour of self-administration on Monday and Friday (the remaining 10 h of self-administration were omitted for the LgA groups on those days). The LgA rats were switched to 4 h daily IVSA sessions for Week 9 and received no self-administration sessions in Week 10.

Non-contingent drug exposure. During Week 9 rats received acute doses of methamphetamine (0.5 mg/kg, i.p.) or oxycodone (0.25 mg/kg, s.c.) immediately prior to the ICSS session to determine if brain reward sensitivity to rewarding drug administration was restored after sustained discontinuation.

Data Analysis. Analysis of the IVSA data was conducted with Analysis of Variance (ANOVA) on the number of infusions earned (ShA: 1 h session; LgA: 11 or 12 h session), on the breakpoints reached in the PR study and on the brain reward threshold (μA). Within-subjects factors of Session, Drug Dose (PR) and/or pre-treatment condition as warranted and between-subjects factors for Access Duration were included. Significant main effects were followed with post hoc analysis using Tukey (multi-level factors) or Sidak (two-level factors) tests to control for multiple comparisons. All statistics were performed in Prism (Graphpad Software, Inc, La Jolla, CA). In all cases, $p<0.05$ was the criterion for statistical significance. Graphs were generated with Prism and/or Excel (Microsoft,

Redmond, WA) and final figures created in Canvas (ACD Systems of American, Inc, Seattle, WA) and/or Adobe Illustrator CS6 (Adobe Systems Inc, San Jose, CA).

Results

Escalation of oxycodone self-administration under extended access conditions

The mean number of intravenous oxycodone infusions obtained by Cohort 1 rats trained under LgA (N=8) conditions was significantly higher compared to infusions by ShA (N=12) rats (**Fig. 1A**). The rmANOVA confirmed

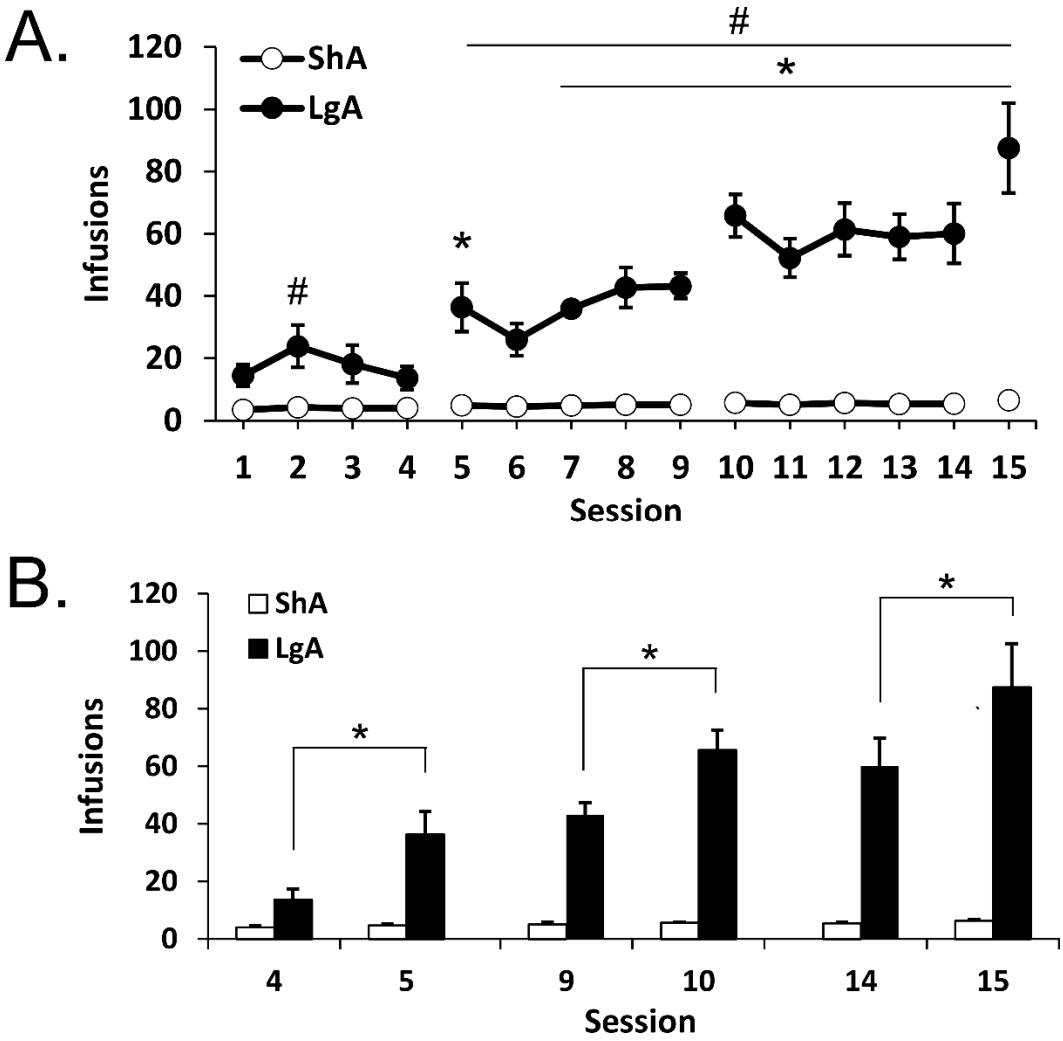

Figure 1. Escalation of Oxycodone Self-Administration Under Extended Access Conditions. Mean (±SEM) infusions for groups of male rats trained to self-administer of oxycodone (0.15 mg/kg/inf) within Long Access (LgA; 12 h) or Short Access (ShA; 1 h) during A) acquisition and B) following weekend abstinence. Broken lines between sessions 4 & 5, 9 &10 and 14 & 15 represent 60 h abstinence period. Significant differences within group from session 1 are indicated by * (unless otherwise indicated) and between group by #.

significant main effects of Session [F(14,252)=24.3; $p<0.0001$], of Access Duration [F(1,18)=97.07; $p<0.0001$], and of the interaction of factors [F(14,252)=21.16; $p<0.0001$]. Post hoc analysis confirmed that significantly more infusions were obtained by LgA rats during sessions 5 and 7-15 compared to session 1, whereas drug intake did not change for the ShA group. LgA rats received significantly more infusions compared to ShA rats during sessions 5-15. During the last session of acquisition, LgA rats and ShA demonstrated 76.9% and 84.47% drug-associated lever responding, respectively. Interestingly, analysis of the sessions before and after the 60 h weekend abstinence periods showed that LgA rats significantly increased drug intake following this extended drug deprivation, whereas ShA showed no significant change (**Fig. 1B**).

The LgA and ShA rats also exhibited group differences during PR dose substitution (**Fig. 2A**). The rmANOVA confirmed significant main effects of Access [F(1,72)=10.77; $p<0.01$] and of Dose [F(3,72)=3.570; $p<0.05$]. Following the 30-day abstinence period, neither LgA (N=7) nor ShA (N=12) rats exhibited any significant change in oxycodone infusions (0.15 mg/kg/inf) obtained under a FR1 response contingency (**Fig. 2B**). One rat (LgA) that completed the acquisition interval was not included post-abstinence due to illness. The ANOVA confirmed a significant main effect of Access [F(1,17)=96.26; $p<0.0001$] and the post hoc analysis confirmed that significantly more infusions were obtained by rats trained under long access compared to short access conditions.

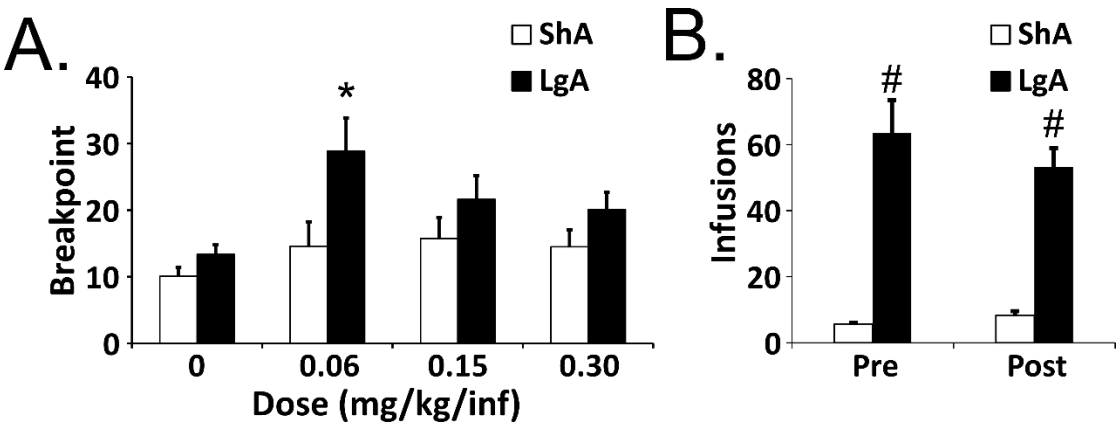

Figure 2. Oxycodone Self-Administration Under Extended Access Increases Motivation for Drug
A) Mean (±SEM) breakpoints reached by oxycodone-trained rats under progressive ratio procedure following training. B) Mean (±SEM) infusions for groups of male rats trained under Long Access (LgA; 12 h) following 30-day abstinence (reflects averages of the 4 sessions immediately before (Pre) and after (Post) the abstinence period). Significant differences within group from session 1 are indicated by * (unless otherwise indicated) and between group by #.

Brain stimulation reward during escalation of oxycodone IVSA

The LgA and ShA rats in the ICSS cohort also exhibited group differences in oxycodone self-administration (**Figure 3**). The ANOVA confirmed significant effects of Session [F(27,351)=3.99; *p*<0.0001], of Group [F(1,13)=19.89; *p*<0.001] and of the interaction of factors [F(27,351)=3.15; *p*<0.0001] on the number of infusions

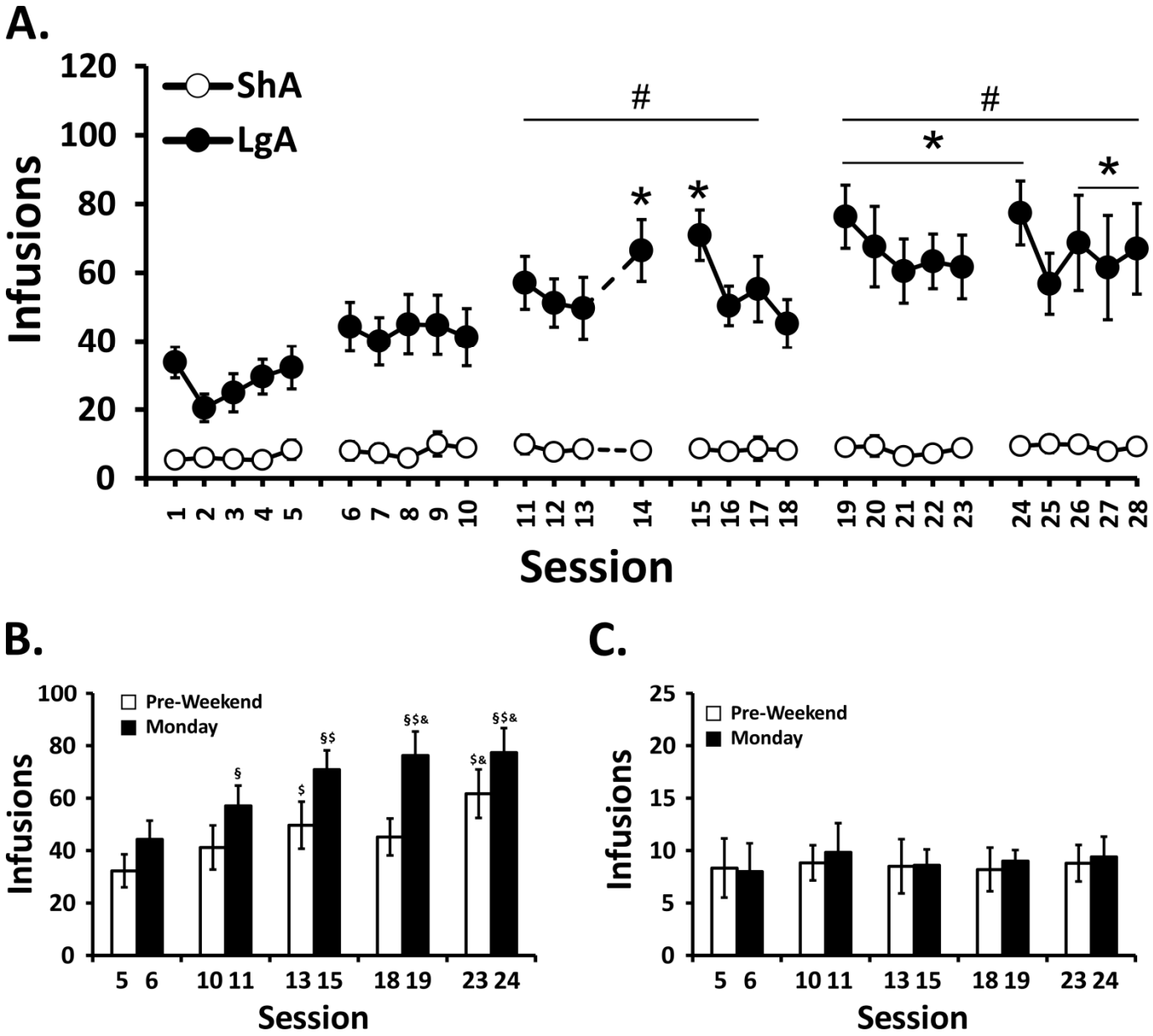

Figure 3. Escalation of Oxycodone Self-Administration in ICSS-trained rats. A) Mean (±SEM) infusions for groups of rats trained to self-administer of oxycodone (0.15 mg/kg/inf) within Long Access (N=10 LgA; 11 h) or Short Access (N=5 ShA; 1 h) sessions following the daily ICSS evaluation. A significant difference from the first session is indicated with * and a significant difference compared with the corresponding day of the ShA group by #. Breaks in the series indicate the weekend and the dotted line indicates a Thursday on which the self-administration session was omitted. Mean infusions for LgA (B) and ShA (C) across weekend breaks. A significant difference across the weekend is indicated with § and significant differences within week from the first week by $ and from the second week by &.

obtained. The post-hoc test confirmed significantly more infusions were obtained by the LgA group in sessions 14, 15, 19-24, 26-28 compared with the first session. In addition, significant group differences were confirmed for sessions 11-17 and 19-28. For LgA rats (N=10) the analysis of the weekend effects confirmed significant effects of Week [F(4,36)=10.23; P<0.0001] and of the Pre-Post weekend factor [F(1,9)=26.56; *p*<0.001] on oxycodone infusions. Post-hoc test confirmed significant increases for each Monday session over the prior end of week session from the 11th session to the 24th.

ICSS thresholds in the LgA group increased across successive days in each week of self-administration but returned nearly to baseline across the weekend breaks (**Figure 4**). The analysis confirmed a significant effect of Day [F(4,36)=9.56; *p*<0.0001] and of Week [F(4,36)=5.91; *p*<0.001] and of the interaction of factors [F(16,144)=2.00; *p*<0.05] on ICSS thresholds. The post-hoc test further confirmed significant increases in each week of self-administration, no change across the abstinence week and differences between days of the self-administration weeks compared with the corresponding weekday of the abstinence week. An attenuation of the ICSS threshold elevation was observed on the 5th day of the third week, following omission of the IVSA session the previous day.

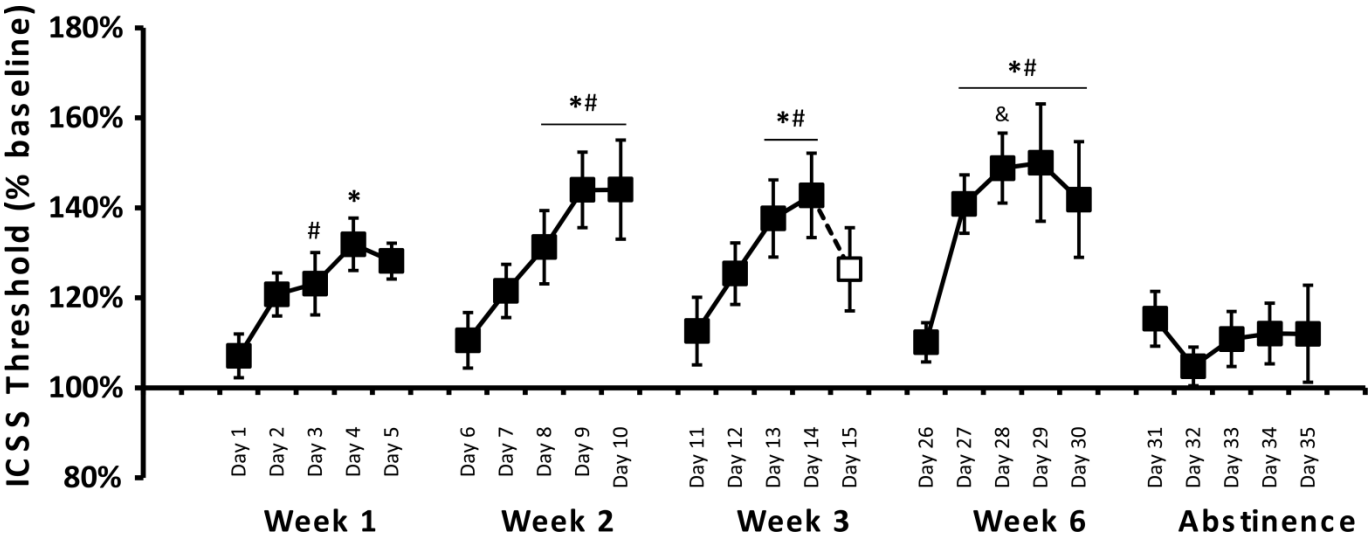

Figure 4. Oxycodone self-administration alters brain reward thresholds. Mean (N=10; ±SEM) ICSS thresholds expressed as percent of the individual baseline threshold. A significant difference from the first day of the week is indicated with * and a significant difference compared with the corresponding day of the Abstinence week by # and from Week 1 by &. The open symbol indicates a Friday preceded by no self-administration on Thursday.

The elevation in reward threshold that was observed later in the self-administration weeks could be reversed with either pre-session injection of oxycodone or by a one hour self-administration session (**Figure 5**). Analysis of the ICSS thresholds before and after a one hour IVSA session on Monday and Friday of Week 8 confirmed a significant effect of the day of the week [F(1,8)=39.29; $p<0.001$] and a significant effect of the pre/post session factor [F(1,8)=5.334; $p<0.05$]. The post hoc test confirmed that pre-IVSA reward thresholds were significantly higher on Friday compared with Monday and that a significant post-IVSA reduction in threshold was observed on Friday.

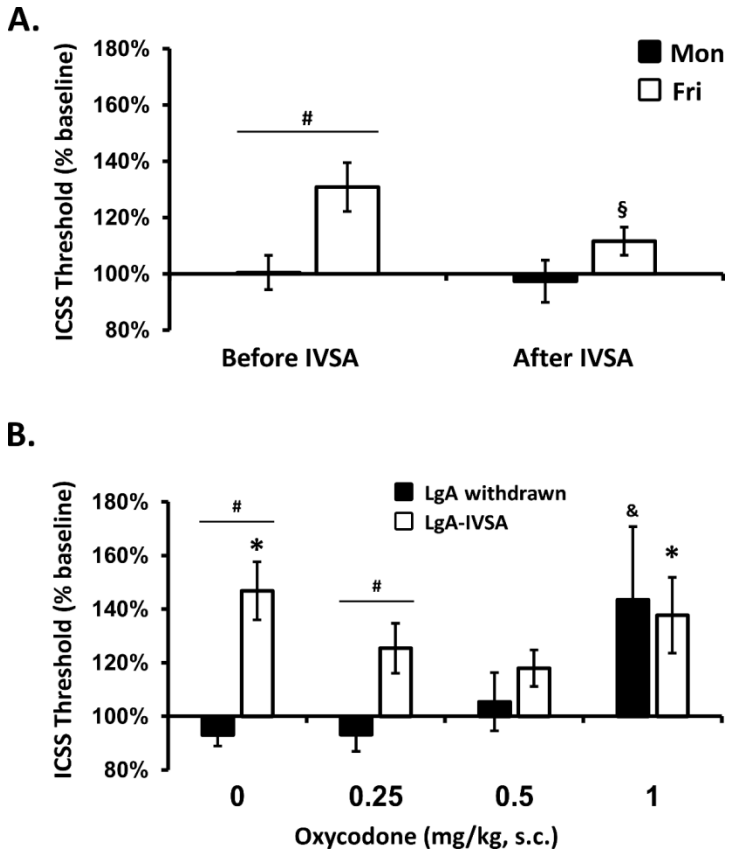

Figure 5. Contingent and non-contingent oxycodone normalizes elevated brain reward thresholds. A) Mean (N=9; SEM) ICSS thresholds before and after self-administration (IVSA) sessions and B) Mean (N=10; SEM) ICSS thresholds in the LgA group expressed as percent of the individual baseline threshold. A significant difference from the baseline is indicated with *, a difference between IVSA and withdrawn dose series by #, a difference from all other s.c. injection conditions by &, a difference before vs. after intravenous self-administration (IVSA) by §.

Following discontinuation of IVSA, additional studies analyzed reward threshold in 7 remaining rats (4 rats were excluded due to unstable ICSS performance) beginning during Week 9 (**Figure 6**). Reward thresholds were significantly reduced 15 min after acute injection of methamphetamine (0.5 mg/kg, i.p.) or oxycodone (0.25 mg/kg. s.c.). Analysis confirmed a significant effect of Drug Treatment [F(1.257,7.541)=8.72; $p<0.05$] and the post hoc test confirmed reward thresholds were significantly lower following injections of both drugs compared to injection of saline.

Discussion

Diversion of prescription opioid medications, such as oxycodone, for nonmedical use and the transition to abuse of illicit opioids has had a tremendous negative impact on US public health and safety over the past two decades. Intravenous self-administration (IVSA) procedures using laboratory rodents have recently been used to model oxycodone-seeking behavior (Ginsburg and Lamb, 2018; Mavrikaki *et al*, 2017), to elucidate the effects of oxycodone abuse on cellular mechanisms and

synaptic function (Blackwood *et al*, 2018; Yuferov *et al*, 2018; Zhang *et al*, 2017; Zhang *et al*, 2018), and to develop and assess potential therapeutic interventions for oxycodone addiction (Neelakantan *et al*, 2017; Nguyen *et al*, 2018; Townsend *et al*, 2017). One prior investigation determined that long (12 h) daily access to oxycodone in IVSA procedures resulted in progressively escalated drug taking compared with 1 h access (Wade *et al*, 2015). In this study, we provide the first confirmation of that finding that animals provided access to oxycodone in 12 h sessions increase their mean intake, whereas animals allowed 1 h access sessions do not. Further, we determined that intermittently increasing the interval of abstinence to 60 hours leads to further increased oxycodone self-administration. So called "deprivation effects" have been previously reported for the self-administration of drugs of abuse. This generally refers to an increase in alcohol or drug consumption after an abstinence interval greater than the usual day-to-day interval. For example, Heyser and colleagues showed that ethanol intake increases after a period of 3-28 days of deprivation compared to baseline (Heyser *et al*, 1997). Such studies have only rarely manipulated the deprivation interval in the course of initial escalation training to determine if the rate of escalation is affected by increasing one or more of the inter-session interval(s). Intermittent periods (24-48 h) of abstinence produced escalation of nicotine intake under extended access conditions (Cohen *et al*, 2012). An increase in nicotine infusions compared to baseline was observed following a single deprivation period, however a pattern of further increase during subsequent sessions preceded by 24-48 h deprivation periods was not observed. Similarly, intermittent access followed by 7 day abstinence during the early stages of cocaine abuse significantly potentiated drug seeking (Calipari *et al*, 2015). This study confirmed that intermittent 60 h deprivations increase oxycodone IVSA, resulting in a step-wise, upward ratcheting pattern of abstinence-induced drug seeking. It is unknown if the deprivation effect in the present data reflect a unique property of oxycodone within the class of opioids or if similar patterns might be observed with e.g., heroin with the appropriate experimental design. These data support an interpretation that oxycodone self-administration under long access conditions is driven in part by increasing negative affect associated with longer durations of drug abstinence.

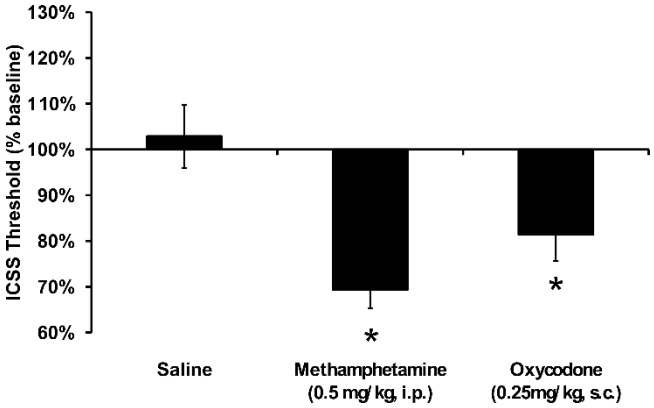

Figure 6. Injection of methamphetamine or oxycodone reduces brain reward threshold. Mean (N=7; ±SEM) ICSS thresholds following challenge with single doses of methamphetamine or oxycodone. A significant difference from Saline is indicated with *.

The increased ICSS thresholds observed during sequential daily extended-access self-administration sessions in this study (these effects were not consistently observed in rats trained to self-administer oxycodone under ShA conditions,data not shown) were consistent with prior results showing brain reward changes following short intervals of drug discontinuation. For example, reward thresholds gradually increased over time during sequential days of 1 hour access MDPV self-administration (Geste *et al*, 2018). More specifically, Jang and colleagues showed that ICSS reward thresholds gradually increase during acquisition of methamphetamine IVSA under extended access conditions but not short access (Jang *et al*, 2013). In both of these studies, the elevation in ICSS reward thresholds may be mediated by the emergence of dependence-related effects during daily abstinence periods (Bruijnzeel *et al*, 2006; Epping-Jordan *et al*, 1998). In a related vein, a period of 23.5 h abstinence following chronic morphine injections reduced ICSS reward (Altarifi *et al*, 2012; Altarifi and Negus, 2011). This interpretation of dependence-related negative affect driving escalated drug taking is further supported by the present data showing that when ICSS thresholds are elevated the self-administration of oxycodone for one hour, or non-contingent injection of an appropriate dose of oxycodone, is sufficient to normalize thresholds back towards baseline (Figure 5). The negative affect hypothesis further holds that as dependence develops, drug taking is no longer as effective as a euphoriant but is only sufficient to *normalize* affect. The present data are also consistent with this prediction since ICSS thresholds were normalized after a moderate dose but were not reduced below baseline following any non-contingent dose across the inverted-U dose-effect function (Figure 5), as was the case for methamphetamine self-administration (Jang *et al*, 2013). Likewise ICSS thresholds were not reduced *below* baseline following one hour of self-administration conducted after 60 hours of discontinuation, i.e., when reward thresholds were normalized. Interestingly, reward thresholds were significantly decreased by methamphetamine or 0.25 mg/kg, s.c., oxycodone after 3-4 weeks of discontinuation from self-administration (Figure 6), demonstrating at least partial recovery of brain reward systems with extended discontinuation of drug access.

The present study also suggests a more nuanced view of the relationship between drug discontinuation, motivation for drug taking and affective state *as indexed by brain reward threshold* is needed. Brain reward thresholds were normalized after 60 hours of discontinuation, while self-administration was increased. This might be viewed as inconsistent with the negative affect hypothesis as an explanation of escalated drug self-administration. However the brain reward thresholds were not *changed* by oxycodone self-administration after 60 hours of discontinuation. This insensitivity to change of brain reward status in the euphoric direction might explain increased self-administration behavior. Similarly, the relatively stable levels of self-administration throughout the

week might reflect the fact that when thresholds were elevated after only 12 hours of withdrawal, one hour of oxycodone self-administration was sufficient to acutely reduce reward threshold. This outcome suggests that it is the relative change in reward threshold subsequent to drug taking that is the more important predictor of self-administration compared with brain reward status at the start of a given self-administration session.

In summary, this study suggests that escalation of oxycodone self-administration under extended access with intermittent abstinence periods may be mediated by both positive and negative reinforcement processes in a time dependent manner. Importantly, these findings may have clinically relevant implications. For example, these data would suggest that individuals on short-term prescribed oxycodone regimens may not wish to skip prescribed days of treatment in an attempt to 'tough it out', as this may in fact lead to increased liability for abuse. These findings highlight the importance of adherence monitoring or adherence enhancing interventions, as non-adherence to pain medication use is very common (Timmerman *et al*, 2016b) and particularly because there does not appear to be an association between medication adherence and pain treatment outcome (Timmerman *et al*, 2016a). Overall, we conclude that drug access and discontinuation intervals each impact the acquisition and maintenance of the self-administration of oxycodone. Escalation of drug intake is associated with extended 60 hour discontinuations and with an insensitivity of brain reward thresholds to oxycodone administration at that time. These data further suggest that a lack of medication adherence may increase a liability for the early stages of oxycodone addiction.

Acknowledgments

This work was funded by support from the United States Public Health Service National Institutes of Health grant R01 DA035281 to M.A.T. The Cohort 1 data were initially described in pre-print form (Nguyen *et al*, 2017b).

Author Contributions

J.D.N. and M.A.T. designed the studies. J.D.N. and Y.G. performed the research and conducted initial data analysis. J.D.N. and M.A.T. conducted the statistical analysis of data, created figures, and wrote the paper. All authors approved of submitted version of the manuscript.

Competing Financial Interests

All authors declare no competing financial interests or potential conflicts of interest.

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