Paradoxical changes in brain reward status during opioid self-administration in a novel test of the negative reinforcement hypothesis

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ABSTRACT

Background and Purpose: The extra-medical use and addiction of prescription opioid analgesics is a growing health problem. To characterize how prescription opioid abuse develops, this study investigated the affective consequences of escalating prescription opioid use using intracranial self-stimulation (ICSS) reward and oxycodone intravenous self-administration (IVSA) models.

Experimental Approach: Male Wistar rats were given access to oxycodone IVSA (0.15 mg/kg/infusion, i.v.) in Short Access (ShA; 1 h) or Long Access (LgA; 12 h) sessions for 5 sessions/week followed by intermittent 60 h discontinuations from drug access, a novel explicit test of the negative reinforcement hypothesis. A separate group was first trained in the ICSS procedure and then in oxycodone IVSA in 11 h LgA sessions.

Key Results: Rats given LgA to oxycodone escalated their responding more than ShA rats, with significant increases following 60 h discontinuations. Pre-session brain reward thresholds increased with sequential daily LgA IVSA sessions, consistent with a growing negative affective state consequent to successive daily intoxication/abstinence cycles. A 1 h oxycodone IVSA interval was sufficient to normalize these elevated reward thresholds, as was, paradoxically, a 60 h weekend abstinence. The increase in ICSS thresholds was attenuated in a group administered the long-acting kappa opioid antagonist norBNI prior to IVSA training.

Conclusions and Implications: Changes in brain reward function during escalation of oxycodone self-administration is driven by an interplay between kappa opioid receptor-mediated negative affective state associated with escalated oxycodone intake and dynamic restoration of brain reward status during longer periods of abstinence.

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 increased by five-fold over the last two decades (CDC, 2016). Despite the growing impact of prescription opioids on public health, relatively few preclinical studies have investigated self-administration of oxycodone, one of the most commonly prescribed medications (OxyContin® or as part of Percocet®). The available preclinical studies have shown that mice self-administer oxycodone intravenously (Zhang, Windisch, Altschuler, Rahm, Butelman & Kreek, 2016), leading to physical dependence and withdrawal (Enga, Jackson, Damaj & Beardsley, 2016). Male and female rats express similar patterns of intake during the early stages of oxycodone self-administration training (Mavrikaki, Pravetoni, Page, Potter & Chartoff, 2017). Male rats trained to self-administer oxycodone under extended daily access conditions (8-12 h) exhibit a progressive escalation of drug intake (Nguyen et al., 2019; Wade, Vendruscolo, Schlosburg, Hernandez & Koob, 2015) similar to heroin escalation under similar extended access conditions (Schlosburg et al., 2013; Vendruscolo, Schlosburg, Misra, Chen, Greenwell & Koob, 2011). Mice also exhibit escalation of oxycodone self-administration under 4 h extended access conditions (Zhang et al., 2014).

The negative reinforcement hypothesis that has been advanced to explain escalating drug IVSA under extended-access conditions (George, Koob & Vendruscolo, 2014; Koob; Koob, 2015; Koob et al., 2014; Lenoir & Ahmed, 2007) holds that the dysphoric, or negative affective, state that is experienced during the daily withdrawal of drug access grows increasingly severe with sequential sessions. The *alleviation* of this dysphoria that is provided by drug access in a subsequent session is therefore the critical stimulus which increases the strength of drug-taking behavior. The intracranial self-stimulation (ICSS) reward procedure has been crucial to the argument that the behavioral escalation phenotype arises from dysregulation of common reward and affective neuronal circuitry and is not merely due to primary pharmacodynamic tolerance. For example, brain reward thresholds progressively increased with

the ongoing self-administration of heroin in 23 h but not 1 h daily sessions (Kenny, Chen, Kitamura, Markou & Koob, 2006). 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, Whitfield, Schulteis, Koob & Wee, 2013) or cocaine under 6 h access (Ahmed, Kenny, Koob & Markou, 2002). Increases persisted for at least 7-8 days post-escalation, with recovery over about 10 days in the methamphetamine study. Withdrawal from ethanol (Schulteis, Markou, Cole & Koob, 1995), nicotine (Epping-Jordan, Watkins, Koob & Markou, 1998), amphetamine (Lin, Koob & Markou, 1999; Lin, Koob & Markou, 2000) and several opioids (Altarifi & Negus, 2011; Negus & Moerke, 2019), including fentanyl (Bruijnzeel, Lewis, Bajpai, Morey, Dennis & Gold, 2006), also elevates reward thresholds in rats. Correspondingly, the somatic signs of withdrawal from heroin increase progressively from 12 to 48 h in adult rats (Doherty & Frantz, 2013). These findings suggest that the motivation for drug-seeking is closely related to brain hedonic state as reflected in ICSS thresholds, and together with the behavior are predictive of abuse-related drug effects (Der-Avakian & Markou, 2012). A study by Wiebelhaus and colleagues showed that acute and repeated injections of oxycodone alter ICSS responding (Wiebelhaus, Walentiny & Beardsley, 2016); however the effects of oxycodone self-administration on ICSS brain reward thresholds have not yet been investigated. Our initial experiment for this study found that 60 h discontinuations of 12 h oxycodone access increased subsequent drug intake more than 12 h discontinuations. This frames a novel test of the negative reinforcement hypothesis through explicit manipulation of the discontinuation interval, which is a major focus of these studies.

The dynorphin / kappa opioid receptor (KOR) system has been shown to be one mechanism critical to the expression of anhedonia, as inferred from the ICSS procedure or from drug self-administration. For example the activation of KORs reduces the rewarding efficacy of brain stimulation (increased ICSS thresholds), whereas blockade of KORs facilitates the reversal of stress- and anhedonia-induced elevation of ICSS thresholds (Chartoff, Sawyer, Rachlin, Potter, Pliakas & Carlezon, 2012; Knoll & Carlezon, 2010; Todtenkopf, Marcus, Portoghese & Carlezon, 2004). A KOR-dependent negative affective state is critical to stress-induced potentiation of drug reward, mediating the consumption, escalation and withdrawal from drugs of abuse (Chavkin & Koob, 2016; Graziane, Polter,

Briand, Pierce & Kauer, 2013; Karkhanis, Holleran & Jones, 2017; Tejeda & Bonci, 2019; Walker & Koob, 2008; Wee & Koob, 2010). More specifically, the long-lasting inactivation of KOR signaling via systemic or intracerebral administration of nor-binaltorphimine (norBNI) attenuates the escalation of heroin (Schlosburg et al., 2013), cocaine (Kallupi et al., 2013; Wee, Orio, Ghirmai, Cashman & Koob, 2009), alcohol (Berger, Williams, McGinnis & Walker, 2013) or methamphetamine (Whitfield et al., 2015) self-administration, under extended access conditions. These results have been interpreted as a consequence of alleviating the negative affective state associated with cycles of drug-taking and (daily) discontinuation.

The present study was designed to determine if the escalation of oxycodone self-administration that is associated with extended (11-12 h) access (Wade, Vendruscolo, Schlosburg, Hernandez & Koob, 2015) is accompanied by decreased brain reward function using the ICSS procedure. The first 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. The second goal was to determine if KOR signaling contributes mechanistically to the development of this negative affective state.

Methods and Materials

Animals. Male Wistar rats (N=58; Charles River, Raleigh, NC) were housed in a humidity and temperature-controlled (23±1ºC) 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). These studies are reported in accordance with the ARRIVE guidelines. Principles of reduction, replacement and refinement were addressed in several ways in this study. Repeated-measures designs

were selected for many studies to minimize the number of groups required and to enhance statistical power for comparisons. In addition, the same group was used in multiple sub-studies, thereby reducing the total number of animals required for the purpose. Subjects were randomly assigned to the major treatment groups within each experiment (Experiments 1, 2 or 3) and procedures conducted in a counter-balanced order. Computer automated data collection (self-administration, ICSS) were conducted in parallel across groups thus no blinding of investigator to treatment group was included in the approach. The experimental apparatus locations were balanced across the groups.

Drugs. Oxycodone HCl was obtained from Sigma-Aldrich (St. Louis, MO) and Spectrum Chemicals (Gardena, CA). Nor-binaltorphimine dihydrochloride (norBNI) and diamorphine HCL (heroin) were obtained from the NIDA Drug Supply Program (Bethesda, MD). All doses are expressed as the salt and were dissolved in physiological saline (0.9% NaCl).

Intravenous Catheterization and Self-Administration. Procedures were adapted from protocols previously described (Nguyen et al., 2019; Nguyen, Grant, Creehan, Vandewater & Taffe, 2017; Nguyen, Hwang, Grant, Janda & Taffe, 2018), and a detailed summary of the experimental design can be found in the **Supplementary Materials**. Rats (Experiment 1) were implanted with chronic indwelling catheters and were then trained daily (Monday-Friday) to self-administer intravenous oxycodone (0.15 mg/kg/infusion; ~0.1 ml/infusion) under Long Access (LgA; 12 h) or Short Access (ShA; 1 h) conditions with 60 h intervals of discontinuation across weekends. Following acquisition, rats were subjected to randomized Progressive-Ratio (PR) dose-response testing wherein doses of oxycodone (0-0.3 mg/kg/infusion) were presented in a balanced order on sequential sessions lasting up to 3 h. Rats were returned to their home cages for an extended 30-day abstinence period and then returned to 12 h sessions to test for re-engagement of drug seeking (re-escalation) following detoxification.

Intracranial Self-Stimulation (ICSS) Reward. For these studies, procedures were adapted from well-established protocols describe (Kenny & Markou, 2006; Kornetsky & Esposito, 1979; Markou & Koob,

1992; Nguyen, Aarde, Cole, Vandewater, Grant & Taffe, 2016). Rats were prepared with unilateral electrodes aimed at the medial forebrain bundle (coordinates: AP -0.5mm, ML ±1.7mm, DV skull -9.5mm). Rats were trained in a procedure adapted from the discrete-trial current-threshold procedure (Kenny & Markou, 2006; Kornetsky & Esposito, 1979; Markou & Koob, 1992; Nguyen, Aarde, Cole, Vandewater, Grant & Taffe, 2016) and were then randomly assigned to ShA (1 h) or to LgA (11 h) self-administration conditions. Rats in Experiment 2 completed daily (M-F) self-administration sessions after ICSS sessions for sequential weeks with weekend discontinuations (60 h). In Experiment 3, a separate group of rats was trained in the ICSS procedure and administered norBNI (30 mg/kg, i.p.; LgA-norBNI) or saline vehicle (LgA-sal) 3 days prior to oxycodone self-administration training. More experimental details can be found in the **Supplementary Materials**.

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 percent change in brain reward threshold (μA) relative to individual baseline. Within-subjects factors of Session, Drug Dose (PR) and/or pre-treatment condition were included as warranted and between-subjects factors for Access Duration and saline/norBNI pretreatment. Significant main effects or interactions 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. Where the data are presented as bar charts, an examination of the individual data did not reveal any unusual aspects of the data that are not made obvious from the bar chart (George et al., 2017).

Results

Escalation of oxycodone self-administration under extended access conditions

In Experiment 1, the mean number of intravenous oxycodone infusions obtained by rats trained under LgA (N=8) conditions was significantly higher than infusions obtained by ShA (N=12) rats (**Figure 1A**). The ANOVA confirmed significant main effects of Session [$F(14,252)=24.3$; $p<0.0001$], of Access

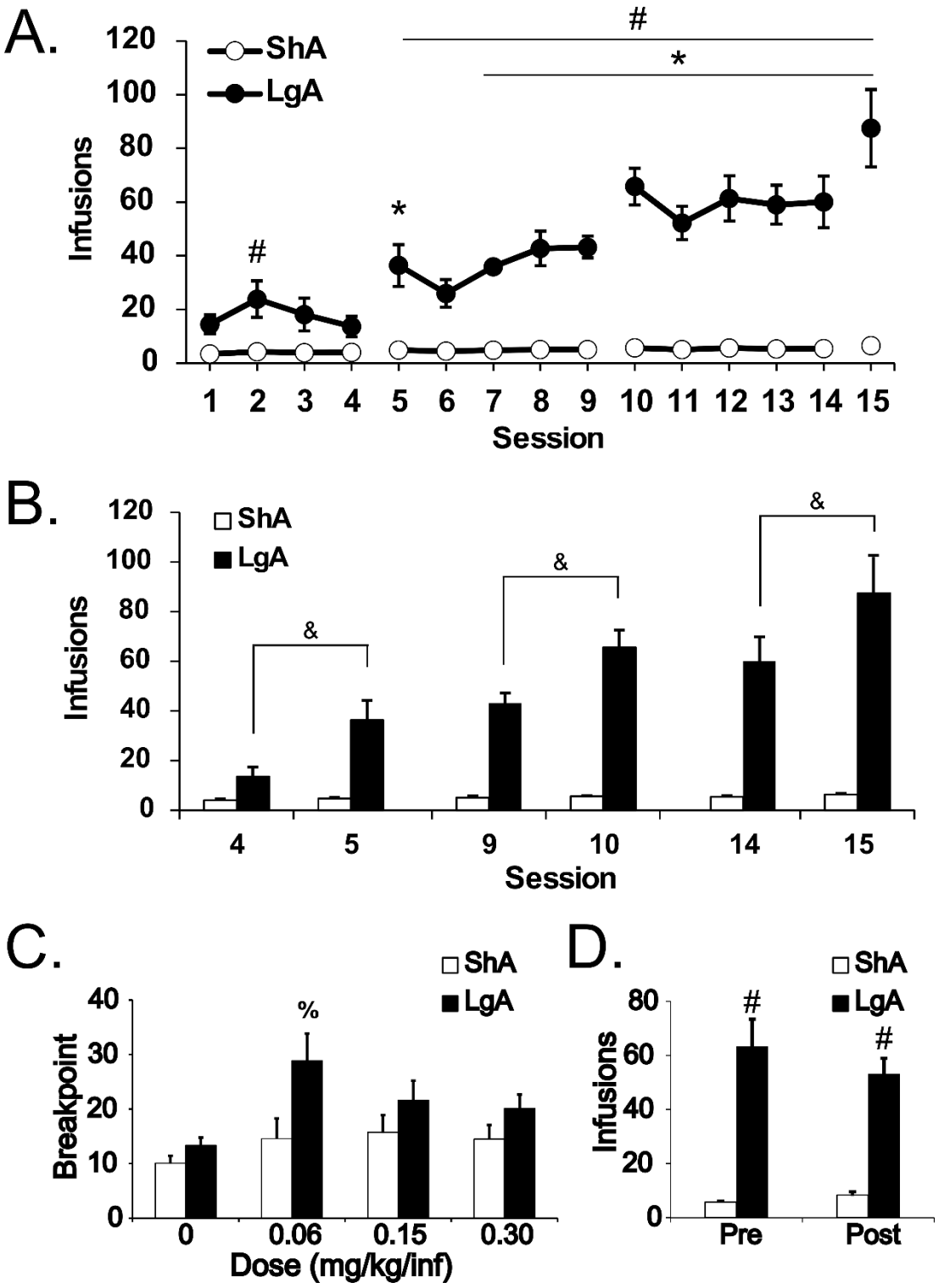

Figure 1. Escalation of Oxycodone Self-Administration Under Extended Access Conditions. *Mean (+SEM) infusions for groups of male rats trained to self-administer of oxycodone (0.15 mg/kg/inf) within Long Access (LgA; 12 h; N=8) or Short Access (ShA; 1 h; N=12) conditions during A) acquisition (broken lines between sessions 4 & 5, 9 &10 and 14 & 15 indicate the 60 h abstinence interval); and B) before and after the weekend abstinence. C) Mean (+SEM) breakpoints reached under the progressive ratio dose-substitution procedure. D) Mean (+SEM) infusions for groups of rats before and after a 30-day abstinence period (reflects averages of the 4 sessions immediately before (Pre) and after (Post) the abstinence). Significant differences within group from session 1 are indicated by * and between group by #. Significant difference between before vs after 60 h abstinence are indicated by &. Significant difference within group from vehicle control is indicated by %.*

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 more infusions were obtained by LgA rats during sessions 5 and 7-15 compared to session 1, LgA rats received significantly more infusions compared to ShA rats during sessions 5-15 and that drug intake did not change across sessions for the ShA group. During the final session of acquisition, LgA rats and ShA exhibited 76.9% and 84.47% drug-associated lever responding, respectively. 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 (**Figure 1B**).

The LgA and ShA rats also exhibited group differences during PR dose substitution (**Figure 1C**) and the ANOVA 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]. 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 (**Figure 1D**) before versus after the 30-day abstinence interval. One rat (LgA) that completed the acquisition interval was not included in the post- abstinence assessment 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 LgA rats on the day after a weekend break.

Brain stimulation reward during escalation of oxycodone self-administration under extended access conditions

The LgA and ShA rats in the Experiment 2 (ICSS-trained) cohort also exhibited group differences in oxycodone self-administration (**Figure 2A**). 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 obtained. The post hoc test confirmed that 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 across weekend breaks (Sessions 11-17 and 19-28) (**Figure 2B**). 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. Whereas for ShA rats (N=5), mean infusions were not significantly different between Pre-weekend and Monday sessions (**Figure 2C**).

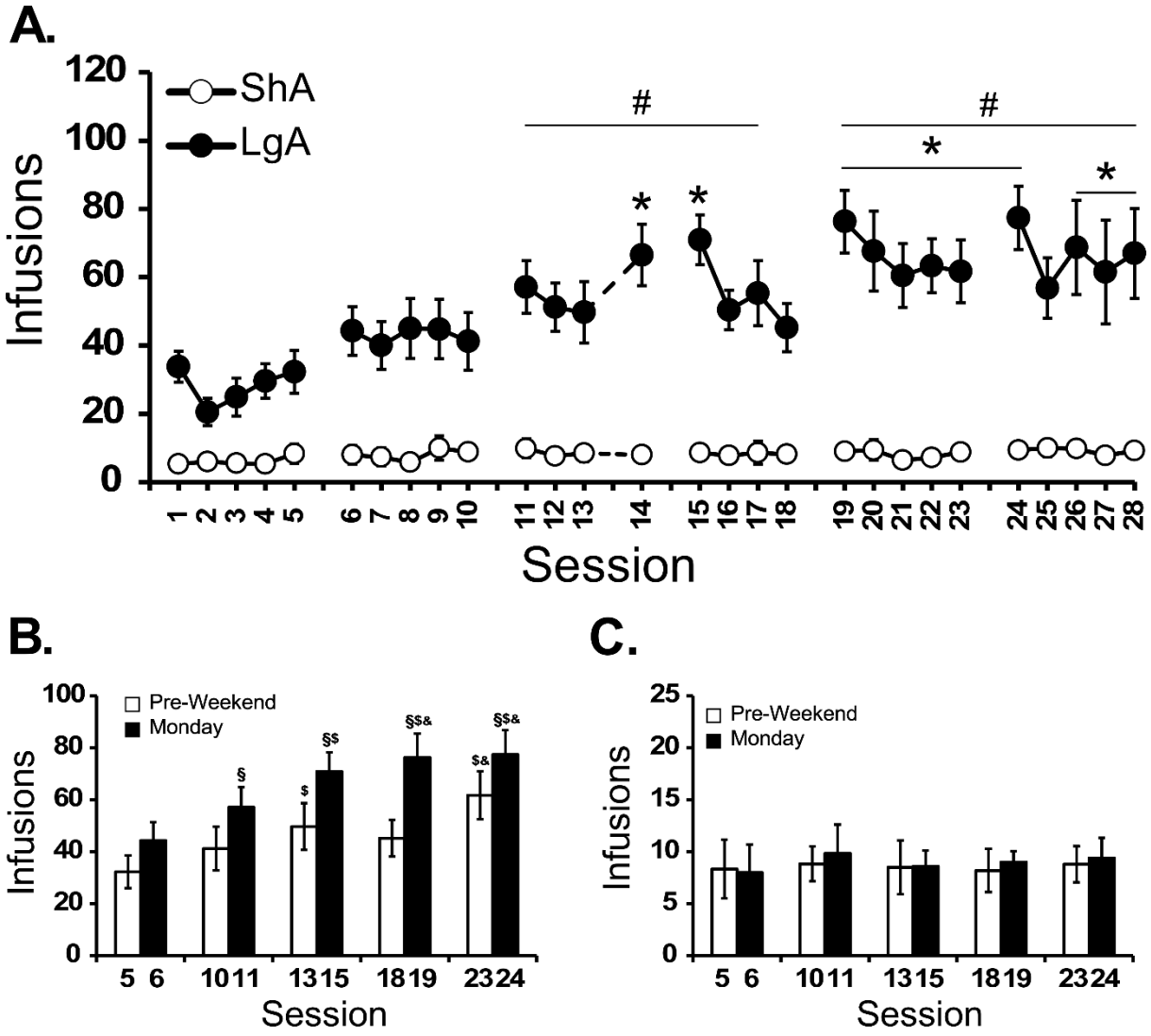

Figure 2. Escalation of Oxycodone Self-Administration in ICSS-trained rats. *A) Mean (+SEM) infusions for groups of rats trained to self-administer of oxycodone (0.15 mg/kg/inf) in Long Access (N=10 LgA; 11 h) or Short Access (N=5 ShA; 1 h) sessions following the daily ICSS evaluation. A significant 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 &.*

ICSS thresholds in the LgA group in Experiment 2 increased across successive days in each week of self-administration but returned nearly to baseline across the weekend breaks (**Figure 3A**). The ANOVA 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 within each week of self-administration, no change within 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 (see **Figure 2A**).

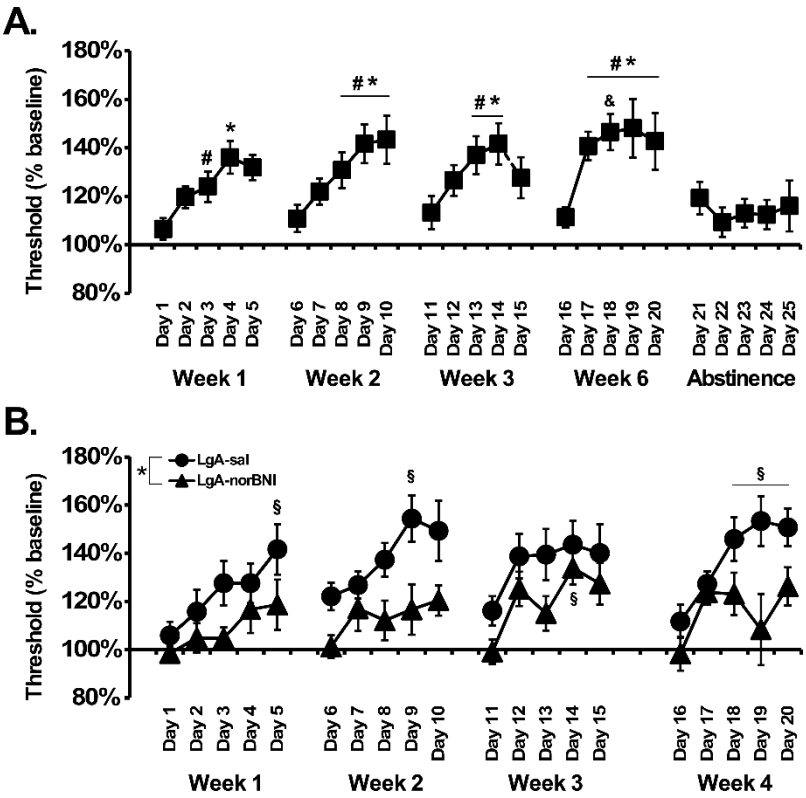

Figure 3. Oxycodone self-administration alters brain reward thresholds partially via kappa opioid receptor signaling. *A) Mean (N=10; ±SEM) ICSS thresholds expressed as percent of the individual baseline threshold. A significant difference from the first day of the week is indicated with * and a significant difference compared with the corresponding day of the Abstinence week by # and from Week 1 by &. B) Mean ICSS thresholds in LgA-sal (N=8; ±SEM) and LgA-norBNI (N=7; ±SEM) rats. A significant within-group difference from the Monday of that week is represented by §. Analysis excluded the week 3 "IVSA" session.*

In Experiment 3, groups of rats (LgA-norBNI) that were injected with norBNI (30 mg/kg, i.p.), a kappa opioid receptor (KOR) antagonist, or saline (LgA-sal), prior to initiating self-administration training were used to determine the effect of KOR function on oxycodone-induced effects on ICSS reward thresholds (**Figure 3B**). Compared to LgA-sal control rats, LgA-norBNI rats had lower ICSS thresholds across successive days (Monday through Friday) during 4 weeks of self-administration. The analysis confirmed a significant effect of Session [F(19,247)=6.949; *p*<0.0001] and of Group [F(1,13)=5.196; *p*<0.05] but not the interaction of factors. Post hoc analysis confirmed ICSS thresholds were significantly increased in LgA-sal rats (Days 5, 9 and 18-20) and LgA-norBNI (Day 14) compared to the first Day of each Week.

We further confirmed in Experiment 3 that the within-week elevations of brain reward thresholds depended on access duration, but not specific opioid identity (**Figure 4A**). ICSS thresholds remained elevated for seven sequential IVSA sessions (Days 16-23) in both LgA-sal and LgA-norBNI rats but returned to the baseline following four consecutive 11 h sessions of saline self-administration (Days 24-27). Analysis confirmed a significant effect of Session [$F(7,91)=11.07$; $p<0.0001$]. Subsequent oxycodone IVSA in 1 h access sessions in Week 6 (Days 28-31) failed to significantly increase reward thresholds. Heroin IVSA under 11 h access duration (Days 32-41) significantly elevated reward thresholds in both groups [$F(4,52)=22.6$; $p<0.0001$] in Week 7 and then in 4 h access sessions in Week 8 [$F(4,52)=2.587$; $p<0.05$]. The Dunnett post hoc test, collapsed across groups, confirmed significant changes relative to pre-saline (Day 23) and the first day of each heroin week, respectively. Group comparisons of daily ICSS

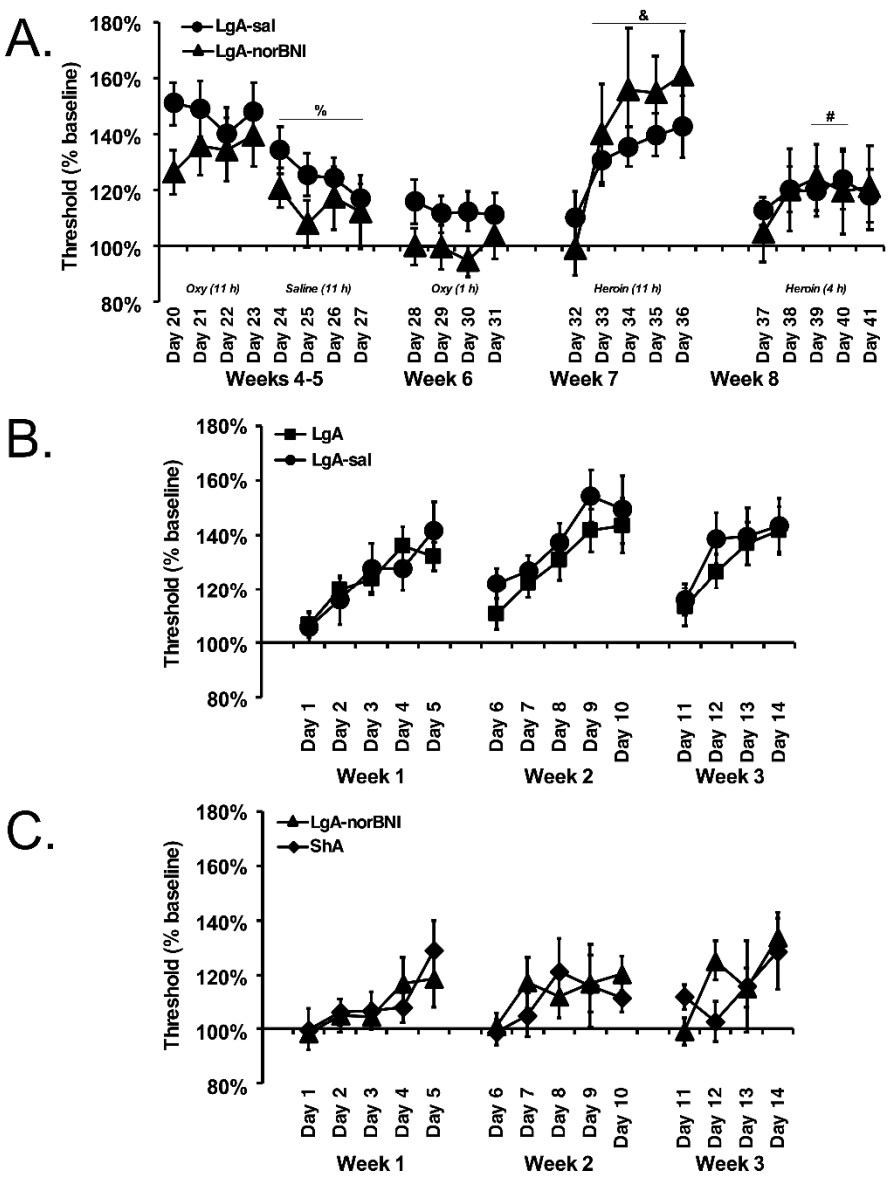

Figure 4. Elevation in brain reward threshold is differentially affected by drug identity and access duration. *A) Mean ICSS thresholds in LgA-sal (N=8 ±SEM) and LgA-norBNI (N=7 ±SEM) rats expressed as percent of the individual baseline threshold. A significant difference, collapsed across group, from Day 23 is indicated with %, from Day 32 with & and from Day 37 with #. Group comparisons of threshold elevations during oxycodone self-administration confirmed that B) the Long Access group from Experiment 2 (LgA; 12 h) and the Long Access group from Experiment 3 (LgA-sal; 11 h; saline pretreatment) group exhibited similar ICSS threshold elevations across the first three weeks of IVSA, and that C) the Short Access (ShA; 1 h) and Long Access group pretreated with nor-BNI (LgA-norBNI; 11 h) exhibited similar ICSS threshold elevations across the first three weeks of IVSA.*

thresholds showed consistency across the LgA (Experiment 2) and LgA-saline (Experiment 3) groups, as they exhibited very similar elevations of reward thresholds across the initial three weeks (**Figure 4B**). More interestingly, the pattern of ICSS threshold increases in the LgA-norBNI (Experiment 3) group was very similar to the pattern of modest increases observed in the ShA (Experiment 2) group(**Figure 4C**).

Normalization of elevated ICSS thresholds by a one hour IVSA session

Reward thresholds were assessed before and after a 1 h self-administration session in LgA groups in Experiments 2 (**Figure 5A**) and 3 (**Figure 5B-D**). In the Experiment 2 group, the analysis of the ICSS thresholds before and after a 1 h oxycodone IVSA session on Monday and Friday 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 5A**). Analysis of ICSS thresholds after a 1 h oxycodone IVSA

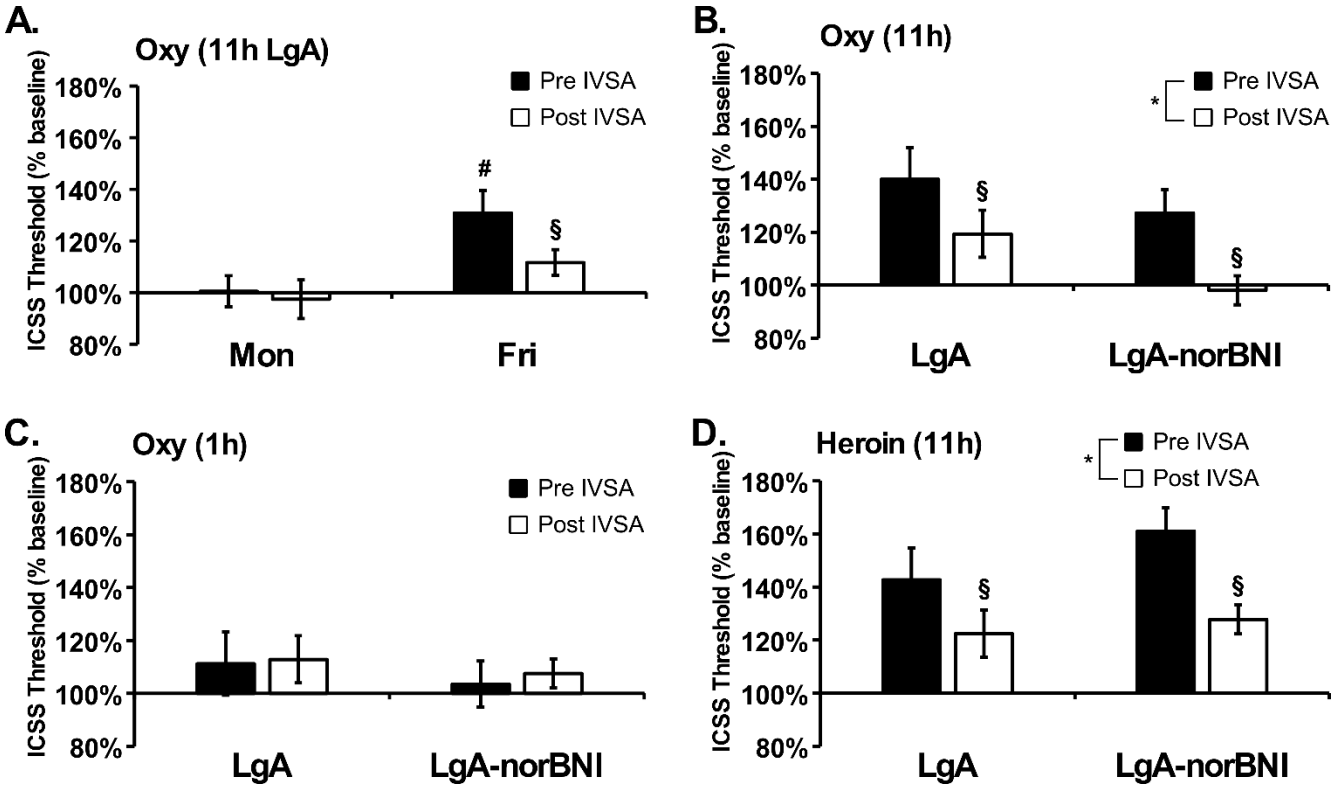

Figure 5. Normalization of ICSS thresholds by 1 h IVSA of oxycodone or heroin. *A) Mean (N=9; ±SEM) ICSS thresholds before and after IVSA sessions. Mean (N=7-8; ±SEM) thresholds before and after B,C) 1 h IVSA of oxycodone or D) 1 h IVSA of heroin in rats trained under 1 and/or 11 h conditions. A marginal mean difference is indicated by *. A significant difference from the pre-IVSA threshold on Monday is indicated by #. A significant difference from the pre-IVSA threshold is indicated by §.*

session in Experiment 3 (**Figure 5B**), confirmed there was a significant effect of Pre/Post Session [$F(1,13)=18.22$; $p<0.001$]. Post hoc analysis further confirmed a significant reduction in ICSS threshold in both LgA and LgA-norBNI groups. In contrast, there was no significant effect of the Pre/Post Session factor during daily 1 h oxycodone IVSA (**Figure 5C**). Finally, there was a significant effect of Pre/Post Session [$F(1,13)=23.16$; $p<0.001$] following 1 h IVSA of heroin during the daily 11 h heroin IVSA training (**Figure 5D**). Post hoc analysis confirmed a significant reduction in ICSS threshold in both LgA and LgA-norBNI groups.

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 & Lamb, 2018; Mavrikaki, Pravetoni, Page, Potter & Chartoff, 2017), to elucidate the effects of oxycodone abuse on cellular mechanisms and synaptic function (Blackwood et al., 2018; Yuferov, Zhang, Liang, Zhao, Randesi & Kreek, 2018; Zhang et al., 2017; Zhang, Liang, Randesi, Yuferov, Zhao & Kreek, 2018), and to develop and assess potential therapeutic interventions for oxycodone abuse (Neelakantan et al., 2017; Nguyen, Hwang, Grant, Janda & Taffe, 2018; Townsend et al., 2017). Prior investigations showed that long (12 h or 3 consecutive 3 h) daily access to oxycodone in IVSA procedures resulted in progressively escalated drug taking compared with shorter (1 h) access (Blackwood et al., 2019; Wade, Vendruscolo, Schlosburg, Hernandez & Koob, 2015). In this study we confirm those findings, since rats provided access to oxycodone in 12 h sessions increased their mean intake across weeks of training, whereas animals allowed 1 h access sessions did not. The dose-response pattern in the PR procedure further confirmed the elevated efficacy of oxycodone as a reinforcer in LgA versus ShA rats, without a change in potency. (This latter finding is consistent with prior investigations of extended-access drug self-administration and is inconsistent with explanations based on pharmacological tolerance where a rightward shift of the dose-effect function would be expected). We discovered that intermittent 60 h

deprivations further increased oxycodone IVSA, producing a step-wise, upward ratcheting pattern of abstinence-related drug seeking (**Figure 1**). The behavioral data support an interpretation that escalated oxycodone IVSA under long access conditions is driven in part by increasing negative affect associated with longer durations of drug abstinence. This represents a novel test of the negative reinforcement hypothesis. Although so called “deprivation effects” have been previously reported for the self-administration of other drugs of abuse under some conditions, such studies have not explicitly manipulated the deprivation interval to determine if the rate of escalation is affected by increasing one or more of the inter-session interval(s). For example, Heyser and colleagues showed that ethanol intake increases after a period of 3-28 days of deprivation compared to baseline (Heyser, Schulteis & Koob, 1997). Intermittent 24-48 h of abstinence produced an escalation of nicotine intake (Cohen, Koob & George, 2012), particularly in the first deprivation period. Intermittent access followed by 7 day abstinence during the early stages of cocaine IVSA significantly potentiated drug seeking (Calipari, Siciliano, Zimmer & Jones, 2015).

The increased ICSS thresholds observed during sequential daily extended-access (LgA) self-administration sessions in this study were consistent with prior results showing brain reward changes following daily 23 h intervals of drug discontinuation. Reward thresholds gradually increased over time during sequential days of 1 h access MDPV self-administration (Geste, Pompilus, Febo & Bruijnzeel, 2018), and during acquisition of methamphetamine IVSA under extended access conditions (Jang, Whitfield, Schulteis, Koob & Wee, 2013); importantly, ICSS thresholds remained significantly elevated over baseline for a week after discontinuation of methamphetamine access in the latter study. In a related vein, a period of 23.5 h abstinence following chronic morphine injections reduced ICSS reward (Altarifi & Negus, 2011). The interpretation that dependence-related negative affect drove the escalated oxycodone IVSA in this study is further supported by the finding that when ICSS thresholds were elevated, the self-administration of oxycodone (or heroin) for one hour (**Figure 5**), or non-contingent injection of an appropriate dose of oxycodone (see **Supplementary Materials, Figure S1**), was able to normalize thresholds towards baseline. Likewise, ICSS thresholds were not reduced *below* baseline following 1 h of self-administration conducted after 60 h discontinuation, i.e., when reward thresholds

were normalized (also see **Figure S1**). This supports the conclusion that as dependence progresses, drug-taking is no longer effective as a euphoriant but is sufficient only to *normalize* affect. This was also the case for ICSS thresholds during extended-access methamphetamine self-administration (Jang, Whitfield, Schulteis, Koob & Wee, 2013). Interestingly, reward thresholds were significantly decreased compared to baseline by oxycodone (0.25 mg/kg, s.c.) or a non-opioid drug, methamphetamine (0.5 mg/kg, i.p.), after 3-4 weeks of discontinuation from self-administration (see **Supplementary Materials, Figure S2**), demonstrating that recovery of brain reward systems is possible with a long interval of discontinuation of drug access.

Perhaps the most interesting, and paradoxical, feature of the present findings was the re-setting of ICSS thresholds to baseline levels, combined with the upward ratcheting of oxycodone IVSA, after 60 h of abstinence. The trend established by 24 h of discontinuation (**Figure 3A**; Day 15) suggests a linear recovery of brain reward, at least across 12-60 h of discontinuation. The failure of 1 h of oxycodone IVSA to alter brain reward on the first session after 60 h discontinuation is an important clue. It implies that self-administration is governed by a *change* in brain reward from an allostatic set-point (Koob, 2015), rather than a fixed target relative to a basal threshold set prior to the onset of drug experience.

Prior work shows that activation of kappa opioid receptor (KOR) signaling elevates ICSS thresholds and decreases motivation to respond for brain stimulation reward (Conway, Puttick, Russell, Potter, Roitman & Chartoff, 2019; Faunce & Banks, 2019; Russell et al., 2014; Todtenkopf, Marcus, Portoghese & Carlezon, 2004). Negus and colleagues demonstrated a lack of effect of the kappa antagonist norBNI on *pain-induced depression* of ICSS (Negus, Morrissey, Rosenberg, Cheng & Rice, 2010); this contrasts with the effect of norBNI pretreatment in the present data where it attenuated *oxycodone-withdrawal-induced elevations* of ICSS brain reward thresholds. It has also been shown that norBNI can attenuate an increase in ICSS thresholds that is caused by withdrawal from daily noncontingent injections of cocaine (Chartoff, Sawyer, Rachlin, Potter, Pliakas & Carlezon, 2012). Interestingly, desipramine normalized ICSS threshold elevations induced by cocaine withdrawal (Markou, Hauger & Koob, 1992), an effect that may be related to the ability of this tricyclic antidepressant to reduce stress-related dynorphin expression (Chartoff et al., 2009) or to directly activate KORs (Onali,

Dedoni & Olianas, 2010). In the present study, brain reward threshold elevations observed in rats treated with systemic norBNI (LgA-norBNI) before the start of LgA IVSA were significantly reduced compared to LgA-sal rats (**Figure 3B**) and paralleled the elevations observed in ShA rats (**Figure 6**). Interestingly, ICSS thresholds in LgA-sal rats were higher at the beginning of each week compared to LgA-norBNI rats, suggesting only a *partial* normalization of reward function after 60 h of discontinuation in the LgA-sal rats. Overall, these data confirm that the increases in ICSS brain reward thresholds caused by sequential days of LgA IVSA of oxycodone are mediated in part by kappa opioid receptor signaling.

The present study suggests that 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 paradoxical, or 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 h 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 h 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 regulator of self-administration compared with brain reward status at the start of a given self-administration session.

Conclusions

In summary, this study suggests that escalation of oxycodone self-administration under extended access with intermittent longer abstinence periods is mediated by negative reinforcement processes in a time-dependent manner. Overall, we conclude that drug access and discontinuation intervals each impact the acquisition and maintenance of the self-administration of oxycodone. Importantly, these findings may have clinically relevant implications. For example, these data would suggest that individuals

on short-term prescribed oxycodone regimens do not 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, Stronks, Groeneweg & Huygen, 2016b) and particularly because there does not appear to be an association between medication adherence and pain treatment outcome (Timmerman, Stronks, Groeneweg & Huygen, 2016a). These data further suggest that a lack of medication adherence may increase a liability for the early stages of oxycodone addiction.

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.

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The authors declare no competing financial interests that influenced the conduct or reporting of this work.

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