

## 1. Title page

# **The Effects of Chronic Naltrexone on Reinstatement of Opioid-Induced Drug-Seeking Behavior and Antinociception**

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## 2. Running Title Page

### **Running Title: Chronic Naltrexone: Opioid-Seeking and Antinociception**

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MOR,  $\mu$ -opioid receptor; OUD, opioid-use disorder; D-E, dose effect;

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### 3. Abstract

Opioid addiction is a chronic relapsing disorder in which drug-seeking behavior during abstinence can be provoked by exposure to a  $\mu$ -opioid receptor (MOR) agonist or opioid-associated cues. Opioid self-administration behavior in laboratory subjects can be reinstated by priming with MOR agonists or agonist-related stimuli, providing a procedure suitable for relapse-related studies. The opioid antagonist naltrexone has been forwarded as a medication that can forestall relapse and, in an extended-release formulation, has demonstrated some treatment success. However, chronic naltrexone treatment has not been extensively investigated in nonhuman subjects and aspects of its pharmacology remain uncertain. For example, the relative effectiveness of naltrexone in reducing the priming strength of opioid agonists differing in efficacy is not well understood. Here, using i.v self-administration and warm-water tail withdrawal procedures, we investigated changes in the direct reinforcing effects of oxycodone and in the priming strength and antinociceptive effects of opioid agonists in squirrel monkeys (n=4) during chronic treatment with naltrexone (0.2mg/kg/day). Results show that naltrexone produced: 1) a ten-fold rightward shift in the dose-response function for the reinforcing effects of oxycodone, and 2) in reinstatement and antinociception experiments, comparable rightward shifts in the dose-response functions for higher-efficacy MOR agonists (methadone, heroin, oxycodone) but rightward and downward shifts in the dose-response functions for lower-efficacy MOR agonists (buprenorphine, nalbuphine, butorphanol). These results suggest that, while chronic naltrexone should be effective in forestalling relapse following exposure to lower- and higher-efficacy agonists, the inability of lower-efficacy agonists to surmount naltrexone antagonism may complicate the prescription of opioids for pain.

#### **4. Significance statement**

Though naltrexone is commonly used in the treatment of opioid use disorder, its ability to reduce the priming strength of opioid agonists has not been extensively investigated. Here we show that chronic naltrexone treatment induces rightward shifts in the reinstatement and antinociceptive properties of higher efficacy opioid agonists, but rightward and downward shifts for lower efficacy opioid agonists, suggesting lower efficacy agonists may not be able to surmount naltrexone-induced antagonism of these two effects and that perhaps naltrexone offers greater protection against lower efficacy agonists.

## 5. Introduction

Naltrexone, a mu-opioid receptor (MOR) antagonist, has been available for the treatment of opioid use disorder (OUD) since 1984. Until 2010, however, its use was limited, in part due to non-compliance leading to high levels of relapse to drug-taking behavior (see Sullivan et al., 2007). In an effort to improve compliance, an extended-release formulation of injectable naltrexone was developed (Vivitrol<sup>®</sup>; Alkermes Inc.), requiring intramuscular injections every 28 days. This regimen provides constant opioid antagonism without the issue of insuring daily compliance and greatly reduced relapse frequency and improved clinical efficacy, yielding rates of abstinence as high as 90% and 50% over 6- and 12-month follow-up periods, respectively (Krupitsky et al., 2011; Krupitsky et al., 2013).

While the extended-release formulation of naltrexone has been a welcome addition to the pharmacotherapies used for OUD, the pressing clinical need for novel medications meant that its relatively rapid introduction into clinical use was not preceded by extensive pharmacological evaluation of its effectiveness. For example, although it is well understood that as a competitive MOR ligand, acute naltrexone antagonism of opioid agonist effects may be surmounted by increases in agonist dose (Withey et al., 2018), the extent or magnitude of chronic naltrexone's protective effects against relapse has not been extensively evaluated (see Lee et al., 2018; Brooks et al., 2010). Such studies are difficult to conduct in human subjects given the limitations of clinical trials, e.g., the inability to prime subjects with an opioid agonist during a period of abstinence. In this regard, previous studies in nonhuman primates have demonstrated that the degree of protection against the relapse-related effects of opioid agonists by buprenorphine, which is also approved for the management of OUD, can vary with the efficacy of the opioid agonist (Withey et al., 2019). In those studies, buprenorphine's protective effects were more readily overcome by higher-efficacy opioid agonists than by lower-efficacy agonists—presumably because lower-efficacy, i.e., partial, agonists were unable to activate a sufficiently large enough population of opioid receptors to fully overcome the protective effects of buprenorphine. A similar

comparison of the relationship, if any, between protective effects of naltrexone against the priming effects of opioids and agonist efficacy has not been conducted.

Another consideration in the use of naltrexone, as well as other pharmacotherapies, for managing OUD is its interaction with prescription opioids that are used for pain management. The use of opioids to provide analgesia in individuals maintained on opioid agonists like buprenorphine or methadone is difficult due to the potential drug interactions as well as uncertainty regarding the extent of opioid tolerance in individual patients (Scimeca et al., 2000; Hoffman et al., 1991). The ability of naltrexone to antagonize all receptor-mediated effects of opioid agonists, including antinociception, suggests that treatment with the extended-release formulation of naltrexone also may complicate pain management with opioid analgesics. In the simplest instance, doses of opioids that usually are prescribed for pain management may be insufficient in patients maintained on the chronic naltrexone regimen (Vickers et al., 2006). Some physicians have attempted to circumvent this issue by prescribing non-opioids such as IV acetaminophen (Piguet et al., 1998) or high dose NSAIDs (McQuay and Moore, 1998) to patients who are maintained on naltrexone. This may be an effective strategy for treating pain in some cases; however, it is of limited value in others, especially in individuals with a long history of OUD who display reduced pain thresholds (Edwards et al., 2016) and might benefit more from treatment with opioid rather than non-opioid analgesics. Yet a reluctance to prescribe higher doses of opioids, even when indicated, is commonplace, in part due to the paucity of data regarding the effectiveness of opioid analgesics during treatment for OUD (Alford et al., 2006).

The present studies in squirrel monkeys were undertaken to provide preclinical information pertinent to both the above concerns—relapse liability and analgesia—that may arise during chronic naltrexone treatment. First, we determined how chronic naltrexone modulates the ability of opioids differing in selectivity and efficacy to reinstate drug-seeking behavior in subjects with a history of oxycodone self-administration. In a second set of experiments, we examined the ability of the same drugs to produce

antinociception in an opioid-sensitive warm-water tail withdrawal assay during chronic naltrexone treatment. Results indicate that the ability of chronic naltrexone treatment to attenuate the relapse-related priming effects and analgesia-related antinociceptive effects of opioids can be overcome by increases in dose but that the magnitude of recovered antinociception may differ depending on pharmacological efficacy.

## 6. Methods

**Subjects.** Eight adult male squirrel monkeys (*Saimiri sciureus*) were housed under a 12-hour light/dark cycle (7 AM to 7 PM) in a climate-controlled vivarium in the McLean Hospital Animal Care Facility. The facility is licensed by the US Department of Agriculture and compliant with guidelines provided by the Committee on Care and Use of Laboratory Animals and described by the Institute of Laboratory Animals Resources, Commission on Life Sciences (National Research Council, 2011). Four subjects participated in IV opioid self-administration studies and the remaining four subjects were used in opioid antinociception experiments. All subjects were fed a high-protein primate chow (Lab Diet Monkey Chow #5040) supplemented with fruit and multivitamins and had unrestricted access to water in the home cage. Food intake was not restricted in the present studies; subjects were weighed daily, and diets were adjusted as needed to maintain stable body weights. Experimental sessions were conducted 5 days/week (Monday to Friday between 8 AM and 6 PM) under protocols approved by the Institutional Animal Care and Use Committee at Mclean Hospital. All subjects had previously participated in studies involving acute injection of drugs from different pharmacological classes but had not received any drugs or participated in any studies for at least 1 month prior to undertaking the present research.

**Apparatus.** Subjects were seated in customized Plexiglas chairs that were situated within ventilated, sound-attenuating enclosures (self-administration studies) or in a small research-purposed room (antinociception studies). The front panel of each chair was outfitted with a pair of colored stimulus lights at eye level and a response lever below each set of lights. One of the two levers was active during the session; each press of the active lever with a force greater than 0.2 N produced an audible click and was recorded as a response. A syringe pump (PHM-100-10; Med Associates, St. Albans, VT) situated outside the front panel could deliver, in self-administration studies, IV injections via an indwelling catheter (see below) or, in antinociception studies, diluted sweetened condensed milk (0.18 ml/delivery) into a custom-designed Plexiglas receptacle (5 x 3.5 x 1.27 cm) between the response levers and easily



accessible to the subject. All experimental events and data collection were controlled by a Med Associates MED-PC software package (Med Associates, East Fairfield, VT).

## **Behavioral Procedures**

### 1. Self-administration and Priming-Induced Reinstatement studies

*Training.* Subjects first were trained to respond on the active lever under a 30-response fixed-ratio (FR30) schedule of milk delivery. Responses on the other lever were recorded but had no programmed consequences. Lever assignments were counterbalanced across subjects and under terminal schedule contingencies, every 30<sup>th</sup> response on the active lever turned off the stimulus lights, delivered the reinforcer, and initiated a short time-out (TO) period of 45 seconds. During the short TO, all stimulus lights were off and responses on either lever had no scheduled consequences. Following this phase of training, indwelling IV catheters were implanted as described below and, subsequently, IV injections of oxycodone (0.1 ml/injection) replaced milk delivery under the FR30 schedule of reinforcement.

*Oxycodone self-administration.* Each subject was prepared with an IV catheter for drug delivery using well established surgical procedures (Platt et al., 2005). In brief, one end of a hydrophilic coated polyurethane catheter (0.381 mm inside diameter, 0.762 mm outside diameter) was inserted and secured into a femoral or jugular vein under isoflurane anesthesia and aseptic conditions. The catheter was threaded subcutaneously to exit the subject's back and, in daily sessions, could be easily accessed and connected to a syringe pump for IV self-administration. When not in use, the externalized catheter was sealed with an obturator and stored in an inside pocket of a nylon jacket worn by the subject.

Following catheterization, stable IV self-administration behavior was developed in all subjects under the FR30 schedule. First, each subject was trained to respond for IV oxycodone (0.01 mg/kg/inj; 0.1 ml/injection) and to extinguish responding when IV saline was available, in daily 90-minute self-administration sessions. When oxycodone consistently maintained responding above vehicle levels, the

dose-response function for oxycodone [0.001–0.1 mg/kg/inj] was established in each subject. The effects of each unit dose and of the saline vehicle were determined for two or more consecutive sessions, until session intake in two consecutive sessions was stable (within 20% of the mean of those session values). Unit doses of oxycodone were studied in an irregular order and once or twice in each subject. The unit dose that yielded the maximum number of IV injections was used as the maintenance dose in IV self-administration sessions during subsequent reinstatement studies. In those studies, oxycodone and saline were available for IV self-administration under a double alternation schedule of sessions (i.e., saline-saline-oxycodone-oxycodone-saline-saline). Reinstatement studies with priming test sessions commenced when stable patterns of baseline performance were evident. Baseline performance for all monkeys was characterized by high levels of responding when the maintenance dose of oxycodone was available for IV self-administration and low levels of responding during sessions of IV saline availability.

*Priming-induced reinstatement.* After oxycodone self-administration studies, the ability of opioids to reinstate IV drug self-administration behavior was determined in four subjects before and during the chronic naltrexone regimen. Test days were the second of two consecutive daily sessions in which IV saline injections were available for self-administration; a selected dose of an opioid—either a full agonist (0.01–0.56 mg/kg heroin, 0.1–1.0 mg/kg methadone, and 0.1–1.0 mg/kg oxycodone) or a partial agonist (0.01–3.2 mg/kg nalbuphine, 0.0032–1.8 mg/kg butorphanol, and 0.001–0.032 mg/kg buprenorphine)—or vehicle was administered intramuscularly (i.m.) 10 minutes prior to the beginning of the 30-min test session. The i.m. route of administration was chosen to distinguish between noncontingent priming injections and response-contingent IV drug or saline injections that could be self-administered during training sessions. For each subject, test sessions were conducted no more frequently than twice per week and only following a session of IV saline availability in which characteristically low levels of responding were observed. The effects of one opioid were fully evaluated in individual subjects prior to initiating tests with another opioid, and the order of testing varied among subjects.

## 2. Opioid antinociception studies

A separate group of four subjects was used to evaluate the antinociceptive effects of opioid agonists in a modified warm-water tail withdrawal procedure described previously (Withey et al., 2018). Subjects first were trained to respond on a lever under a fixed-ratio 10-response (FR10) schedule of food reinforcement (0.15 ml of 30% sweetened condensed milk in water). Under this schedule, completion of the FR10 while red stimulus lights were illuminated on the front panel turned off the lights, triggered milk delivery, and initiated a TO period of 30 seconds during which responding had no scheduled consequences. A 20-second limited hold was imposed on the FR10 schedule requirement; thus, the elapse of 20 seconds before the completion of 10 responses turned off stimulus lights and initiated the 30-second TO but did not trigger milk delivery. Experimental sessions comprised four or five sequential cycles, each consisting of a 10-min TO component, during which no lights were on and responding had no programmed consequences, followed by a 5-min response component, during which the FR schedule contingencies were in effect. This protocol permitted cumulative drug dosing during successive cycles of test sessions and injections were administered shortly after the onset of each 10-minute TO. To study antinociceptive effects of drugs, the latency to withdraw the tail from warm water was measured during each of the 30-second TO periods of the 5-min response components. In brief, the distal 2 inches of the subject's tail was immersed in water (35, 50, 52, or 55°C) and the latency to withdraw the tail from the water was recorded. The withdrawal of the subject's tail from the water or the elapse of 10 seconds terminated each trial; in the latter case, the experimenter removed the tail from the water to avoid potential tissue damage (10-sec cutoff). The order of water temperature presentations within and across components was randomized, with the provisos that determinations of tail withdrawal latency at 50, 52, or 55°C occurred no more than once in each 5-minute response component and only following the recording of control values for 35°C water. This latter provision ensured that the subsequent withdrawal of the tail from a higher temperature of water likely reflected a nociceptive response. The limited hold contingency of the schedule of food reinforcement ensured that the number of determinations per component ranged from 6 to 10, depending on the number

of reinforcement deliveries during the component. Data from sessions in which sequential injections of i.m. saline were administered across components provided vehicle control values. Training and test sessions typically comprised four or five components but were terminated if response rates during the preceding response component were below 0.2 responses per second.

## Data Analysis

*Self-administration and reinstatement studies.* The number of responses and self-administered injections in Experiment 1 were recorded in all experimental sessions and total session intake was calculated as the product of unit dose and number of injections. Overall rates of responding (responses per second) were calculated for each cycle by dividing the number of lever presses emitted in the presence of stimulus lights by the time during which the stimulus lights were illuminated. Group means  $\pm$  S.E.M. for all endpoints were calculated by averaging mean values for individual monkeys and were analyzed using Student's t-test or ANOVA followed by Dunnett's or Bonferroni's multiple comparison tests, as appropriate, and for all analyses significance was set at  $p < 0.05$  (Prism version 9.4.0, GraphPad Software, San Diego, CA).

*Opioid antinociception studies.* Overall rates of responding (responses per second) were calculated for each cycle by dividing the number of lever presses emitted in the presence of stimulus lights by the time during which the stimulus lights were illuminated. Individual mean control values were calculated by averaging response rates obtained during four components of control sessions in which sequential injections of saline were administered. Statistical analyses (e.g., calculation of 95% CI) were conducted with doses expressed as log transformed values.  $ED_{50}$  values were calculated by linear interpolation for increases in tail withdrawal latency and decreases in response rate after agonist administration. The  $ED_{50}$  for decreases in response rate for each drug was divided by the  $ED_{50}$  for the drug's effect on tail withdrawal latency to provide an index of the behavioral selectivity of its antinociceptive effects versus

its behaviorally disruptive effects. Group ED<sub>50</sub> ratios for each behavioral measure were calculated from the average of individual ED<sub>50</sub> values.

## Drugs

*Chronic Naltrexone.* The daily dosage of naltrexone during the chronic regimen was 0.2 mg/kg delivered over 24 hr and, based on body weight, was selected to reflect a pharmacotherapeutic dosage of naltrexone provided by Vivitrol®, the extended-release formulation of naltrexone (380 mg/28 days). To mimic the extended-release formulation, iPrecio programmable minipumps (SMP-200; Alzet iPrecio, Cupertino, CA) delivered a constant infusion of 8.33 µg/hr naltrexone via an indwelling subcutaneous (SC) hydrophilic coated polyurethane catheter (0.55 mm inside diameter) that previously was inserted and secured to the latissimus dorsi muscle under isoflurane anesthesia and in aseptic conditions. The catheter exited the subject's back in the scapular region on the side opposite the IV catheter exit site (described below). A 22G stainless steel connector was used to attach the externalized portion of the SC catheter to the iPrecio pump, which was protected in an inside pocket of a nylon jacket worn by the subject at all times. The pump was refilled every third day and replaced when the battery expired. Daily treatment with 0.2 mg/kg/day naltrexone lasted for 5 months for reinstatement studies and for 3 months for antinociception studies.

*Opioid agonists.* Both agonists with high MOR efficacy (i.e., the 'full' agonists methadone, heroin, oxycodone; Morgan and Christie, 2011) and lesser MOR efficacy (i.e., the 'partial' agonists buprenorphine, butorphanol, nalbuphine; Cook et al., 2000) were used in the present studies. Oxycodone hydrochloride and nalbuphine hydrochloride were purchased from Sigma/RBI (Natick, MA). Heroin hydrochloride and buprenorphine hydrochloride were obtained from the National Institute on Drug Abuse (Rockville, MD). Methadone hydrochloride and butorphanol tartrate were obtained from Eli Lilly (Indianapolis, IN) and Bristol Laboratories (Evansville, IN), respectively. Drugs were diluted to desired

concentrations in sterile 0.9% saline and filter-sterilized using a 0.22-mm Millipore filter (Millipore Corporation, Billerica, MA). Drug doses are expressed in terms of free base weights. Drugs administered intramuscularly (i.m.) were injected into the calf or thigh muscle in volumes of 0.3 ml/kg of body weight or less. In both self-administration and antinociception experiments, the order of drugs tested varied among subjects.

## 7. Results

*Oxycodone Self-Administration:* Oxycodone self-administration was successfully established in all subjects. As shown in Figure 1, the relationship between unit dose and the number of injections during the 90-min session is described by an inverted U-shaped function characteristic for IV self-administration behavior under simple fixed-ratio schedules of reinforcement. Before chronic treatment with naltrexone, a unit dose of 0.01 mg/kg oxycodone maintained the highest level of responding on the injection-lever, resulting in a mean ( $\pm$  SEM) value of  $26.3 \pm 4.2$  injections per session (left panel, open symbols). Fewer injections were self-administered ( $18.4 \pm 6.5$  and  $17.1 \pm 2.8$  injections) when lower (0.0032 mg/kg/inj) and higher (0.032 mg/kg/inj) unit doses of oxycodone, respectively, were available for IV self-administration; self-administration behavior occurred only at vehicle levels when the unit dose of oxycodone was further lowered to 0.001 mg/kg. In contrast to the inverted U-shaped relationship between the number of self-administered injections per session and unit dose, total drug intake increased monotonically as a function of unit dose. Intake of the peak unit dose of 0.01 mg/kg/inj oxycodone during the 90 min session averaged  $0.26 \pm 0.04$  mg/kg whereas IV self-administration of the tenfold higher unit dose of oxycodone, 0.1 mg/kg/inj, yielded an approximately 3 to 4-fold higher intake over the course of the session ( $0.85 \pm 0.2$  mg/kg; Figure 1, right panel, open symbols).

The peak number of self-administered injections of oxycodone was not greatly altered during chronic naltrexone treatment ( $26.3 \pm 4.2$  vs  $23.3 \pm 1.8$  injections, respectively;  $t_{(12)}=0.58$ , n.s.). However, the continuous presence of naltrexone resulted in a ten-fold rightward shift in the dose-effect function for the number of self-administered injections of oxycodone during the daily session (Figure 1, left panel, closed symbols), reflected in a 10-fold increase in the unit dose of oxycodone required to achieve peak levels (from 0.01 to 0.1 mg/kg/inj). As a consequence of the increase in unit doses of self-administered oxycodone without concomitant decreases in injection frequency, the intake of oxycodone increased in the presence of naltrexone. For example, session intake of 0.1 and 0.32 mg/kg/inj increased approximately 2.5 and 4-fold, respectively, over values for the highest self-administered IV dose of

oxycodone during prechronic dose-effect determinations (0.1 mg/kg/inj;  $F_{(7,50)}=49.6$ ,  $p<0.01$ ; see Figure 1, right panel, closed symbols).

*Reinstatement of IV self-administration behavior by opioid agonists.* Prior to chronic treatment with naltrexone, one or more pre-session priming doses of each opioid full and partial agonist provoked IV self-administration behavior in each subject. These data are reflected in changes in both the number of saline injections (top panels) and response rates (bottom panels), which varied as a function of priming dose for each opioid (Figures 2 and 3, open symbols). The magnitude of these effects did not greatly vary among drugs, regardless of efficacy at the MOR ( $F_{(7,50)}=0.38$ , n.s.). For example, optimal doses of the higher efficacy, i.e., full, agonists (methadone, heroin, and oxycodone) led to 11-13 saline injections and response rates of 0.25-0.45 responses per second (Figure 2, open symbols) similarly, optimal doses of the lower-efficacy, i.e., partial, agonists (buprenorphine, butorphanol and nalbuphine) led to 12-16 saline injections and response rates of 0.4-0.5 responses per sec (Figure 3, open symbols). Fewer injections of IV saline and, as well, lower response rates followed i.m. administration of lower or higher doses of each priming drug.

During chronic treatment with 0.2 mg/kg/day naltrexone, dose-response functions for priming effects and response rate shifted 3 to 10-fold rightward for all agonists (Figure 2, closed symbols). Peak numbers of saline injections during test periods were self-administered following doses of 1.0, 0.56 and 1.0 mg/kg of methadone, heroin and oxycodone, respectively, all significantly higher than the doses that produced peak effects in the prechronic condition ( $F_{(11,24)}=23.2$ , Bonferroni adjusted  $p\leq 0.015$ ). Notably, the magnitude of priming effects ( $12.7 \pm 4.9$ ,  $12.0 \pm 1.0$ ,  $10.7 \pm 1.8$  injections for, respectively, methadone, heroin and oxycodone) was quantitatively comparable to that recorded prior to chronic naltrexone ( $F_{(11,24)}=0.47$ , n.s.). As with the higher-efficacy agonists, dose-response functions for the priming effects of all three lower-efficacy opioid agonists were shifted 3 to 10-fold rightward during chronic treatment with 0.2 mg/kg/day naltrexone. The magnitude of the priming effect of nalbuphine was only slightly lower during,



compared to before, chronic naltrexone treatment ( $11.7 \pm 2.9$  vs.  $12.7 \pm 3.8$  injections, respectively); however, the mean number of saline injections following optimal doses of buprenorphine (0.01 mg/kg) and butorphanol (0.1 mg/kg) decreased from pre-chronic values (i.e., from  $12.3 \pm 1.9$  to  $6.3 \pm 2.0$  injections, and  $15.7 \pm 2.3$  to  $10.3 \pm 5.9$  injections, respectively). Correspondingly, average response rates also were shifted rightward and/or downward for buprenorphine, butorphanol, and, to a much lesser extent, nalbuphine (Figure 3, bottom panels, closed symbols). These differences were not detected by ANOVA ( $F_{(11, 24)}=0.56$ , n.s.) likely reflecting individual variability in the dose at which peak effects occurred.

*Antinociceptive and response rate-decreasing effects of opioid agonists.* Figures 4 and 5 show opioid dose-response functions for tail withdrawal latency (top panels) and rates of food-maintained responding (bottom panels) before and during chronic naltrexone; Table 1 shows  $ED_{50}$  values derived from these data. Prior to chronic treatment, each of the opioid full agonists (methadone, heroin and oxycodone; Figure 4) and partial agonists (buprenorphine, butorphanol, nalbuphine; Figure 5) produced graded decreases in rates of operant responding and increases in tail withdrawal latency at each water temperature (50, 52, and 55°C). The magnitude of antinociceptive effects was related to water temperature for all opioids except methadone. Thus, the highest dose of all three full agonists eliminated operant responding but only methadone (1.0 mg/kg) produced maximal (10-sec) increases in the mean tail withdrawal latency at all three temperatures. The highest doses of heroin and oxycodone (0.32 and 0.56 mg/kg, respectively) produced maximal effects only in 50°C and 52°C water and only  $6.7 \pm 0.59$  and  $6.9 \pm 1.0$  sec increases in tail withdrawal latency from water heated to 55°C water, respectively. Among partial agonists, the highest cumulative doses of buprenorphine and butorphanol (0.1 and 0.32 mg/kg, respectively) but not nalbuphine (3.2 mg/kg), also eliminated operant behavior. Like the full agonists, buprenorphine increased the mean latency to tail withdrawal to 10-sec in 50°C and 52°C water and produced somewhat lesser antinociceptive effects in 55°C water (mean latency of  $8.0 \pm 1.1$ -sec).

Following the highest dose of butorphanol, mean latencies to tail withdrawal were 10-sec,  $7.4 \pm 1.3$ -sec, and  $4.6 \pm 1.9$ -sec in water heated to, respectively, 50, 52, and 55°C. The highest dose of the MOR partial agonist nalbuphine, which markedly reduced but did not eliminate operant responding, produced lesser antinociception than other opioid agonists, with mean latencies to tail withdrawal of  $7.4 \pm 1.8$ -sec in 50°C water,  $5.4 \pm 2.4$ -sec in 52 and  $5.8 \pm 2.1$ -sec in 55°C water.

During chronic naltrexone treatment, dose-response functions for the effects of opioid full and partial agonists on food-maintained behavior shifted ~3 to 10-fold rightward in a parallel manner, and correspondingly higher doses of each drug were required to disrupt operant performance. Thus, the highest doses of the full agonist methadone (1.8 mg/kg) and the partial agonist nalbuphine (3.2 mg/kg) produced >50% reductions in response rate whereas the highest doses of the full agonists heroin (1.8 mg/kg) and oxycodone (5.6 mg/kg) and of the partial agonists buprenorphine (0.32 mg/kg) and butorphanol (1.0 mg/kg) eliminated operant responding. Dose-response functions for the antinociceptive effects of both full and partial agonists also shifted rightward or, for nalbuphine, downward. Excepting methadone and nalbuphine, the magnitude of the antinociceptive effects of the highest doses of all opioids under the several temperature conditions was unchanged from comparable prechronic values. Although the highest dose of methadone (1.0 mg/kg) before the chronic naltrexone regimen produced maximal antinociceptive effects at all water temperatures, the highest dose of methadone during the chronic regimen (1.8 mg/kg) sufficed to produce a maximal antinociceptive effects only in 50°C water; mean latencies to tail withdrawal from 52°C and 55°C water were approximately 8 and 4 sec, respectively. In contrast, regardless of water temperature, the antinociceptive effects of nalbuphine plateaued at a low level during the chronic naltrexone regimen and, even in 50°C water, no dose of the partial agonist up to 10 mg/kg increased the mean latency to tail withdrawal to as much as 4 sec.

The ratio of ED<sub>50</sub> values for disrupting operant behavior and producing antinociception was calculated for each water temperature and provides a measure of behavioral selectivity at each water temperature. Thus,

ED<sub>50</sub> ratios among the three full agonists before chronic naltrexone treatment ranged from 0.92 to 1.37 when the nociceptive stimulus was least intense (50°C) but only from 0.39 to 0.78 when the nociceptive stimulus was most intense (55°C). Similarly, ED<sub>50</sub> ratios for the three partial agonists ranged from 1.23 to 2.31 in 50°C and, in 55°C water, could only be computed for buprenorphine (0.71) because the limited antinociceptive effects of butorphanol and nalbuphine were too low for ED<sub>50</sub> calculations. The effects of chronic naltrexone treatment on ED<sub>50</sub> ratios differed among opioids. Thus, ED<sub>50</sub> ratios for the full agonists during the chronic regimen were not greatly changed from prechronic values, ranging from 0.97 to 1.39 in 50°C water and from 0.38 to 0.43 in 55°C water. Similarly, ED<sub>50</sub> ratios for butorphanol did not change or only increased during the chronic naltrexone regimen. On the other hand, ED<sub>50</sub> ratios for buprenorphine, which were >1 in 50°C and 52°C water prechronically, decreased to 0.53 and 0.71, respectively, during the chronic regimen. Moreover, ED<sub>50</sub> ratios during the chronic naltrexone regimen could not be calculated for nalbuphine due to insufficient antinociception at any of the three water temperatures.

## 8. Discussion

Oxycodone engendered IV self-administration behavior in all subjects, characterized by the inverted-U relationship between unit dose and the number of self-administered injections that is generally observed under fixed-ratio schedules of reinforcement. The numbers of injections of oxycodone at the peak of the bitonic dose-effect function and on its descending limb in the present single-lever study were comparable to those in a previous choice study in which IV oxycodone injections and sweetened condensed milk were concurrently available for self-administration (Withey et al., 2019). Of interest, the lowest unit dose of oxycodone that was reinforcing here, 0.003 mg/kg, was not self-administered under choice conditions in the earlier study, reflecting its lesser reinforcing strength in the presence of a competing reinforcer. In reinstatement experiments, priming with opioid agonists consistently elicited IV self-administration behavior, albeit leading only to injections of saline.

Acute naltrexone has been shown to produce rightward shifts in dose-response functions for heroin self-administration, reflecting decreases in the potency of MOR reinforcers and indicative of surmountable antagonism (Rowlett, et al., 1998). Here, the dose-response function for oxycodone self-administration also shifted rightward during chronic naltrexone. These data most likely reflect the continuing ability of naltrexone to block MORs during chronic treatment, which also is the likely mechanism underlying naltrexone's clinical efficacy in the treatment of opioid use disorder. Such blockade by naltrexone, in turn, prevents the activation of MORs by oxycodone or other opioid agonists until the agonist concentration is high enough to successfully compete with naltrexone for occupancy—here, empirically defined by the full re-emergence of oxycodone's reinforcing effects. The ability of oxycodone to fully surmount naltrexone's antagonistic effects contrasts with data from previous studies involving chronic treatment with the MOR partial agonist buprenorphine (0.32 mg/kg/day; Withey et al., 2019). Thus, levels of oxycodone self-administration behavior were similar in the presence and absence of naltrexone (peaks of ~25 injections per session) whereas peak levels of oxycodone-maintained responding decreased

by approximately 50% during chronic buprenorphine treatment (from ~35 to ~18 injections per session). This difference between the chronic treatment regimens is further illustrated by the comparison of changes in total session intake of peak reinforcing doses of oxycodone under the differing conditions. For example, session intake of the peak unit dose of oxycodone during chronic naltrexone (0.1 mg/kg/inj) was ~8-fold greater than intake of the peak unit dose prechronically (0.01 mg/kg/inj) but only ~5-fold greater when subjects were treated daily with buprenorphine (Withey et al., 2019).

Chronic treatment with naltrexone also attenuated the priming effects of opioid full and partial agonists, which comports nicely with early reports showing that acutely administered naltrexone can antagonize the reinstatement of opioid self-administration in laboratory animals (deWit and Stewart, 1983) and more recent reports that chronic treatment with the partial agonist buprenorphine also may attenuate priming effects of opioid agonists (Withey et al., 2019). As discussed above for oxycodone's reinforcing effects, qualitative differences were evident in the attenuation of opioid priming effects by chronic naltrexone and in earlier studies with buprenorphine. Chronic naltrexone treatment here led to rightward shifts in the dose-response functions for priming-induced injections with all full opioid agonists and nalbuphine, consistent with competitive antagonism. In contrast, chronic buprenorphine treatment led to a rightward and downward shift in the dose-response functions for priming-induced injections with the same opioid agonists (Withey et al., 2019). Taken together, these observations support the view that chronic naltrexone and chronic buprenorphine attenuate the reinforcing and relapse-related effects of opioid agonists via qualitatively different mechanisms of action, i.e., competitive antagonism by naltrexone vs. pseudo-irreversible partial agonism by buprenorphine (Walker et al., 1995; Withey et al., 2018).

The ability of opioids to produce antinociception has been related both to stimulus intensity, e.g., water temperature, and opioid efficacy (Morgan et al., 1999). Both factors were evident in the present results. For example, in prechronic dose-response determinations, 1.0 mg/kg of methadone, the highest-efficacy agonist studied here, eliminated responding and produced maximal increases in tail withdrawal latency in

all water temperatures. In contrast, doses of the other full, albeit lesser-efficacy, agonists that eliminated responding were maximally effective in 50°C or 52°C water but were less effective at the highest water temperature (55°C). In the same vein, the partial agonists buprenorphine and nalbuphine were more effective in 50°C and/or 52°C water than in 55°C water, whereas butorphanol was maximally effective at 50°C, less effective when the temperature was raised to 52°C, and least effective when the temperature was highest (55°C).

As with their priming strength, the antinociceptive effects of opioid agonists and their disruptive effects on operant performance were attenuated by chronic treatment with naltrexone. Dose-response functions for each agonist, excepting nalbuphine, were consistently shifted rightward by chronic naltrexone, indicative of surmountable antagonism. Based on changes in ED<sub>50</sub> values, the magnitude of antagonism ranged from approximately 2-fold for methadone's antinociceptive effects in 50°C water to approximately 13-fold for oxycodone's antinociceptive effects in 55°C water or butorphanol's rate-decreasing effects. In the latter regard, the increase in the ED<sub>50</sub> value for the rate decreasing effects of butorphanol represented the largest antagonism by naltrexone of the rate-decreasing effects of opioids in the present studies. Inspection of the butorphanol dose-response functions (Figure 5) shows that cumulative doses of butorphanol up to 0.32 mg/kg, which initially produced graded rate-decreasing effects, were without effect in the presence of naltrexone, whereas the next cumulative dose of butorphanol, 1.0 mg/kg, eliminated responding. Presumably, the increase in dosage, i.e., from 0.32 to 1.0 mg/kg butorphanol, produced MOR-mediated actions that sufficed to eliminate responding. On the other hand, butorphanol is a mixed-action mu/kappa partial agonist (Picker et al., 1996) and, as kappa-mediated actions are less sensitive to antagonism by naltrexone than MOR-mediated actions, it is also possible that kappa-mediated actions contributed to the pronounced effects of the highest dose of butorphanol on responding (and, correspondingly, its effects on tail withdrawal latency) during the naltrexone regimen.

Excepting nalbuphine, opioid agonists generally retained their antinociceptive effects during chronic treatment with naltrexone, albeit at higher doses required to surmount naltrexone's antagonistic actions. Nalbuphine, like butorphanol, is considered a mixed-action mu/kappa partial agonist with low efficacy at both types of opioid receptor (Walker and Young, 1993; Gerak et al., 1994; Walker et al., 2004). Consistent with this characterization, even the highest dose of nalbuphine (10 mg/kg), which reduced food-maintained responding, produced very little antinociception during the chronic naltrexone regimen. Although these findings are consistent with nalbuphine's inability to surmount antagonistic effects of chronic buprenorphine (Withey et al., 2019), they are somewhat surprising in light of the parallel rightward shift in the dose-response functions for nalbuphine's priming effects during chronic naltrexone treatment. The development of substantial tolerance to nalbuphine's opioid actions is well-documented (Walker et al., 1997; Gringauz et al., 2001) and, possibly, the earlier administration of opioids in the present studies led to a persisting tolerance and/or cross-tolerance to its antinociceptive effects. However, nalbuphine produced dose-related decreases in food-maintained responding during the same sessions in which it did not greatly increase tail withdrawal latency, which does not easily support this explanation. It also may be that the efficacy requirement for nalbuphine's antinociceptive effects is higher than for its priming or response rate-reducing effects, and that the doses of nalbuphine that surmounted antagonism of its latter effects did not sufficiently activate the fraction of the MOR population necessary for antinociception.

ED<sub>50</sub> ratios for the opioid agonists obtained before the chronic naltrexone regimen generally agree with previously published values (Withey et al., 2018; 2019) and indicate very little, if any, behavioral selectivity in their antinociceptive effects. Doses of opioids that increased the latency to tail withdrawal in the lowest temperature of water (50°C) were not substantively lower than those that disrupted operant performance, yielding ED<sub>50</sub> ratios that, with few exceptions, approximated 1. Moreover, opioids were less potent in the highest temperature of water (55°C), yielding ED<sub>50</sub> ratios that, without exception, were below 1 and, in most cases, below 0.5. ED<sub>50</sub> ratios for either full or partial opioid agonists were not

systematically altered by chronic naltrexone and, excepting the 60% increase in the ED<sub>50</sub> ratio for butorphanol in 50°C water, were either similar to or decreased from prechronic values. In most cases, decreases in ED<sub>50</sub> ratios during the chronic naltrexone regimen were minor (<2-fold); however, ED<sub>50</sub> ratios were reduced by >2-fold for both methadone in 55°C water and buprenorphine in 50°C water, reflecting considerably greater antagonism by naltrexone of their antinociceptive effects than their response rate-decreasing effects. Overall, the effects of the chronic naltrexone regimen on ED<sub>50</sub> ratios did not differ qualitatively from those of chronic treatment with buprenorphine (Withey et al. 2018; Table 1). Although differences in chronic treatment (naltrexone vs. buprenorphine) perhaps contributed to differences in the magnitude of decrease in ED<sub>50</sub> ratios in the two studies, the similarity in the overall effects of chronic treatment with naltrexone and buprenorphine, i.e., decreases in ED<sub>50</sub> ratio, is notable. In conjunction, these results highlight the challenge of identifying opioid drugs or treatment conditions that preserve antinociceptive effects independently of their ability to disrupt behavioral performance or provoke drug-seeking behavior.



## 9. Acknowledgements

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## 10. Data availability statement

The authors declare that all the data supporting the findings of this study are contained within the paper.

## 11. Authorship contributions

Participated in research design: Withey, Bergman, Paronis

Conducted experiments: Withey

Performed data analysis: Withey

Wrote or contributed to the writing of the manuscript: Withey, Bergman, Paronis

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No author has an actual or perceived conflict of interest with the contents of this article.



## 14. Figure Legends

Figure 1. Intravenous (IV) oxycodone self-administration before (open symbols) and during (closed symbols) chronic naltrexone treatment (n=4 monkeys). The left and right panels show, respectively, the number of self-administered oxycodone injections (left panel ordinate) and total intake (right panel ordinate) during the 90-min session as a function of unit dose of oxycodone. Abscissae: unit dose of oxycodone available for self-administration.

Figure 2. Reinstatement of IV self-administration behavior by the opioid full agonists methadone (left), heroin (middle) and oxycodone (right) before and during chronic naltrexone treatment (n=3 monkeys). Top panels show the number of saline injections self-administered over the 30-minute session, and bottom panels show response rates. Abscissae: dose (mg/kg) administered as an i.m. priming injection before the session. Ordinate: number of saline injections (top) and response rates (bottom).

Figure 3. Reinstatement of IV self-administration behavior by the opioid partial agonists buprenorphine (left), butorphanol (middle) and nalbuphine (right) before and during chronic naltrexone treatment (n=3 monkeys). Top panels show the number of saline injections self-administered over a 30-minute session, and bottom panels show response rates. Abscissae: dose (mg/kg) administered as an i.m. priming injection before the session. Ordinate: number of saline injections (top) and response rates (bottom).

Figure 4. Effects of the opioid full agonists methadone, heroin, and oxycodone on the latency to tail withdrawal from water (top panels) and food-maintained responding (bottom panels). Abscissae: dose (mg/kg) of opioid administered cumulatively before sequential session components; ordinates: latency (sec) to withdraw tail from water (50°, 52° or 55° C; top panels) and response rate (responses/second; bottom panels). Other details as in Figure 1.

Figure 5. Effects of the opioid partial agonists buprenorphine, butorphanol and nalbuphine on the latency to tail withdrawal from water (top panels) and food-maintained responding (bottom panels). Abscissae: dose (mg/kg) of opioid administered cumulatively before sequential session components; ordinates: latency to withdraw tail from water (50°, 52° or 55° C; top panels) and response rate (responses/second; bottom panels). Other details as in Figure 1.

## 15. Tables

**Table 1**

ED<sub>50</sub> values for each agonist's antinociceptive and behaviorally disruptive effects, and ED<sub>50</sub> ratios of antinociceptive effects to response rate disruptive effects. ED<sub>50</sub> values given are group means ± S.E.M. in milligrams per kilogram and were determined from interpolation of individual (n = 4) dose-response functions.

	Behavior disruption ED <sub>50</sub>		Antinociception ED <sub>50</sub>					
	Prechronic	Chronic	50°C		52°C		55°C	
			Prechronic	Chronic	Prechronic	Chronic	Prechronic	Chronic
<b>Methadone</b>	0.30 ± 0.06	1.0 ± 0.25*	0.32 ± 0.06	0.72 ± 0.19	0.30 ± 0.07	1.20 ± 0.25*	0.38 ± 0.04	2.62 ± 1.55*
<i>ED<sub>50</sub> ratio</i>			0.92	1.39	0.97	0.83	0.78	0.38
<b>Heroin</b>	0.10 ± 0.02	0.54 ± 0.28	0.08 ± 0.03	0.49 ± 0.06*	0.07 ± 0.02	0.54 ± 0.20*	0.18 ± 0.04	1.25 ± 0.46*
<i>ED<sub>50</sub> ratio</i>			1.37	1.11	1.41	1.01	0.58	0.43
<b>Oxycodone</b>	0.16 ± 0.04	1.47 ± 0.53*	0.14 ± 0.03	1.51 ± 0.23*	0.22 ± 0.03	2.30 ± 0.32*	0.41 ± 0.09	5.28 ± 1.88*
<i>ED<sub>50</sub> ratio</i>			1.09	0.97	0.73	0.64	0.39	0.28
<b>Buprenorphine</b>	0.01 ± 0.002	0.05 ± 0.04*	0.009 ± 0.001	0.10 ± 0.02*	0.01 ± 0.002	0.08 ± 0.02*	0.03 ± 0.01	0.19 ± 0.14*
<i>ED<sub>50</sub> ratio</i>			1.23	0.53	1.04	0.71	0.45	0.29
<b>Butorphanol</b>	0.03 ± 0.01	0.38 ± 0.06*	0.02 ± 0.007	0.18 ± 0.04*	0.05 ± 0.02	0.54 ± 1.58	NA	NA
<i>ED<sub>50</sub> ratio</i>			1.35	2.17	0.52	0.7	NA	NA
<b>Nalbuphine</b>	0.58 ± 0.25	NA	0.25 ± 0.17	NA	NA	NA	NA	NA
<i>ED<sub>50</sub> ratio</i>			2.31	NA	NA	NA	NA	NA

\* Indicates significant differences between prechronic and postchronic ED<sub>50</sub> values, based on no overlap in 95% CI.

Figure 1

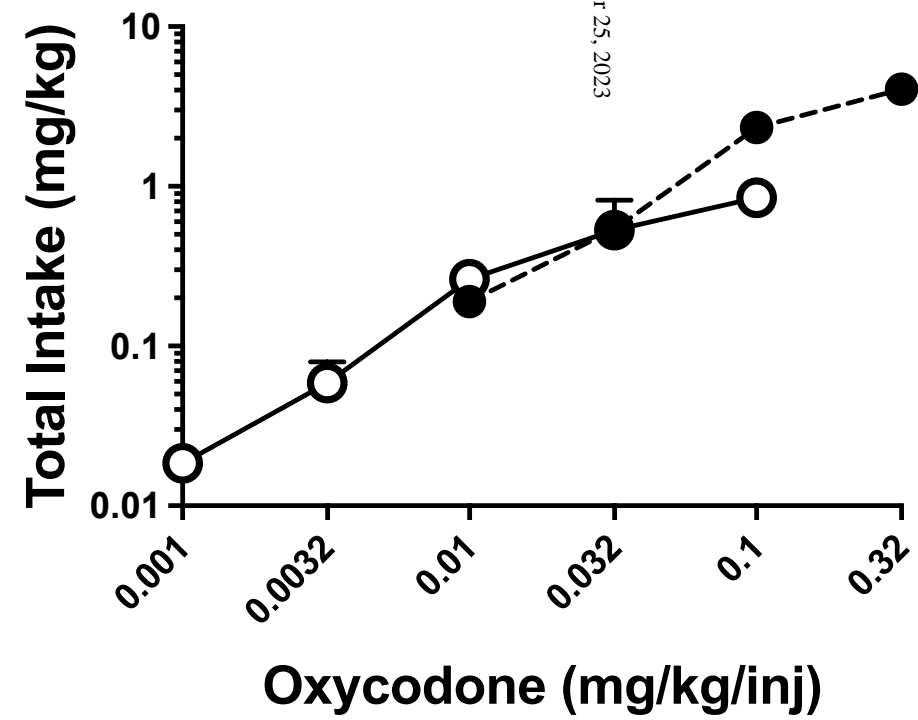
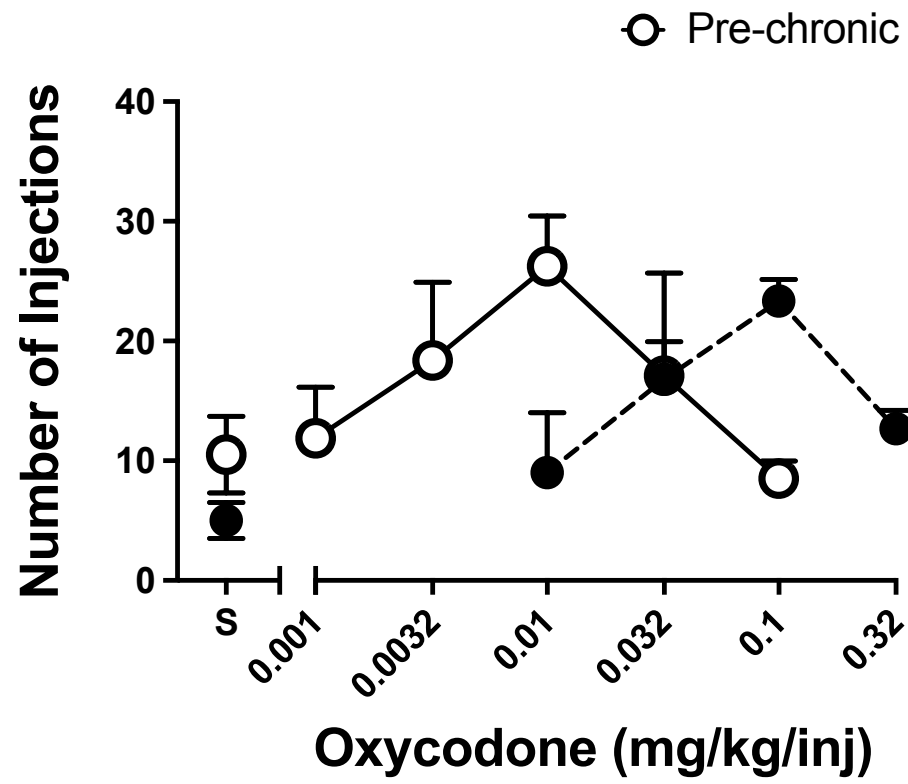


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Figure 2 - Chronic naltrexone

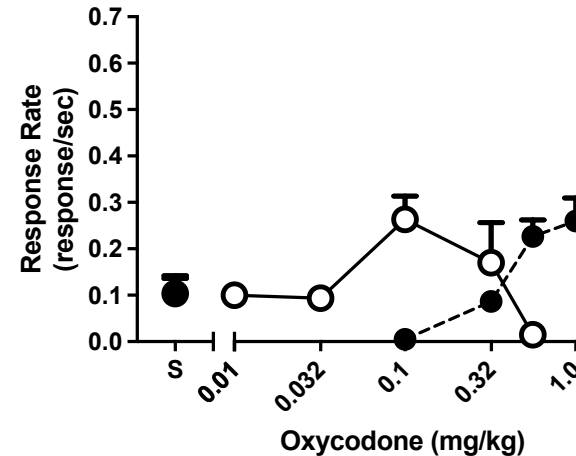
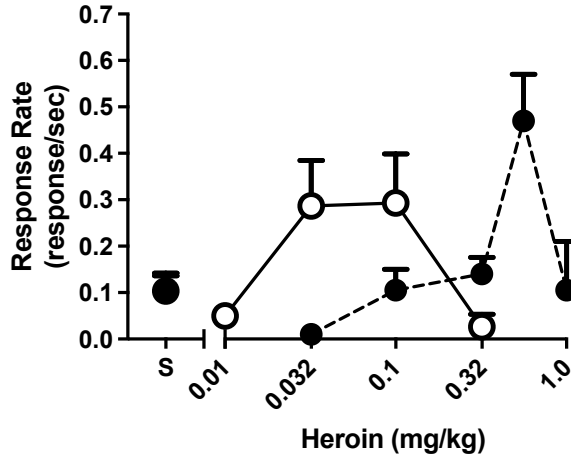
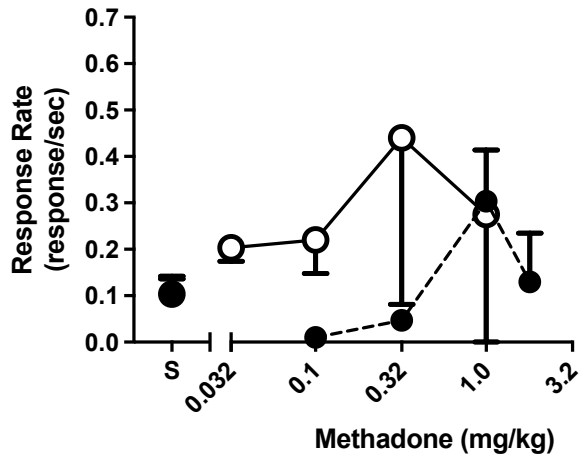
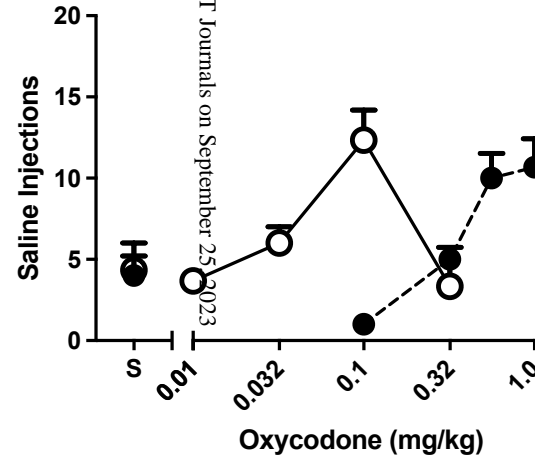
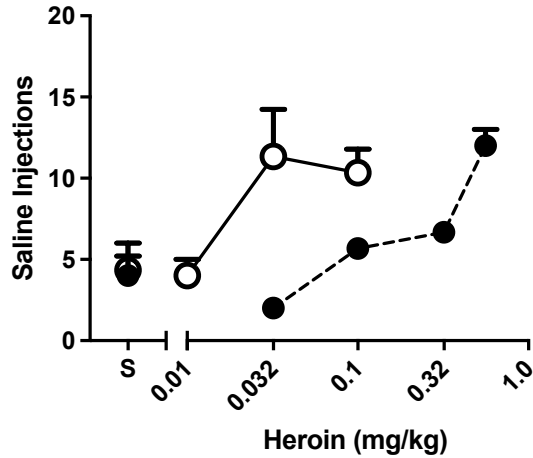
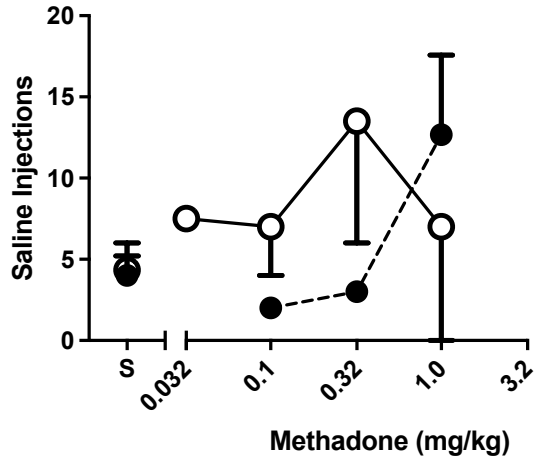
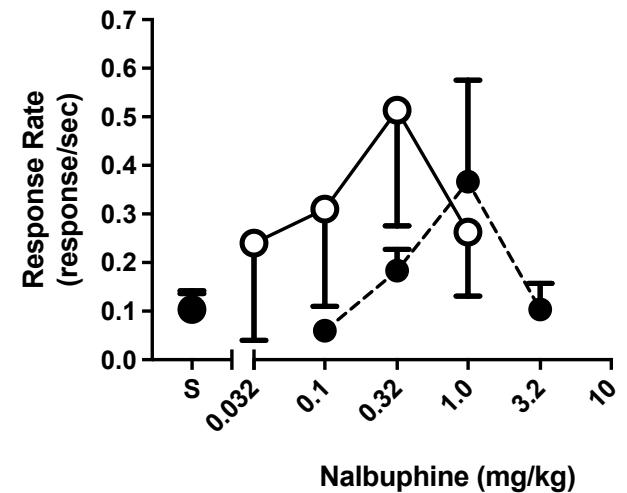
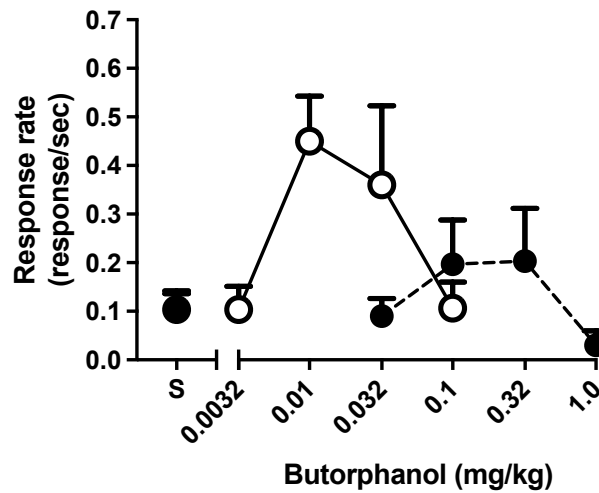
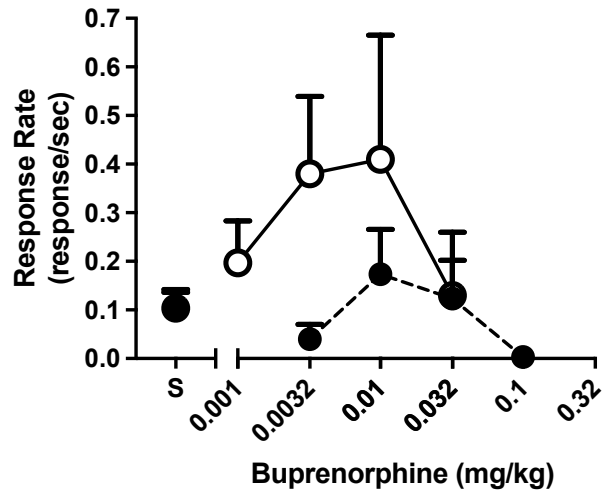
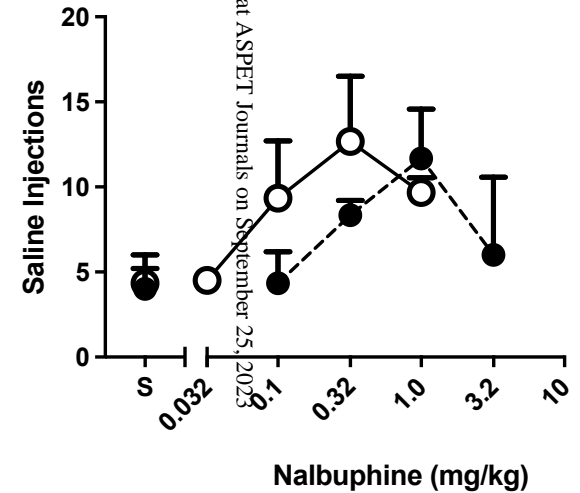
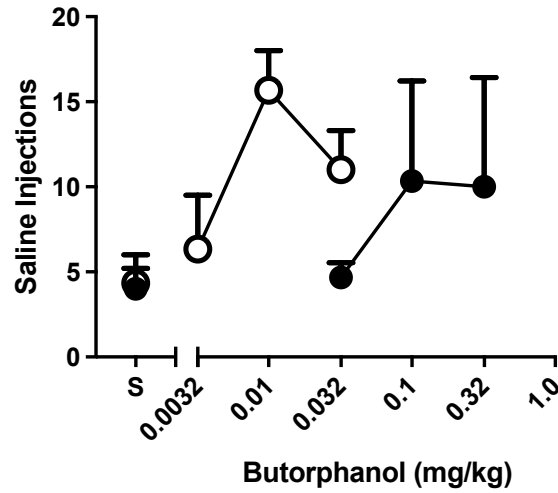
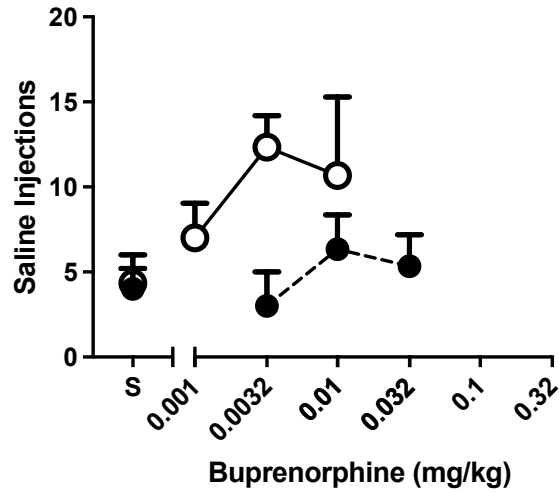
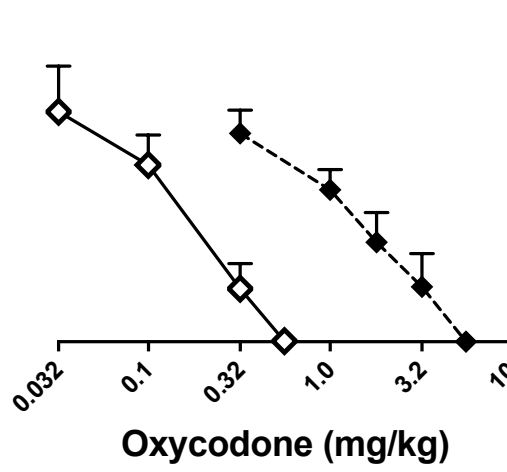
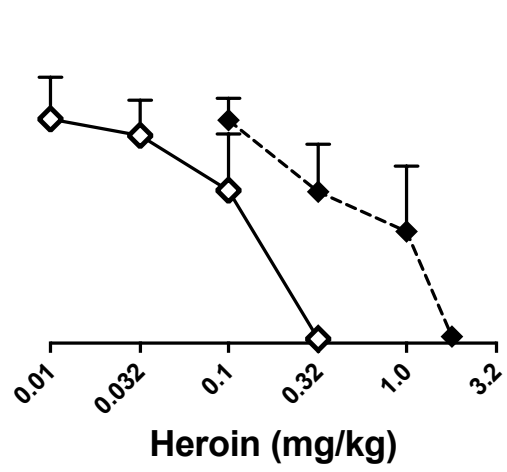
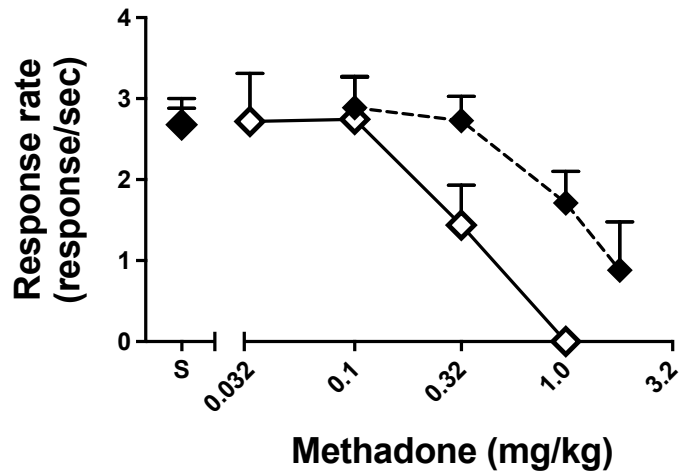
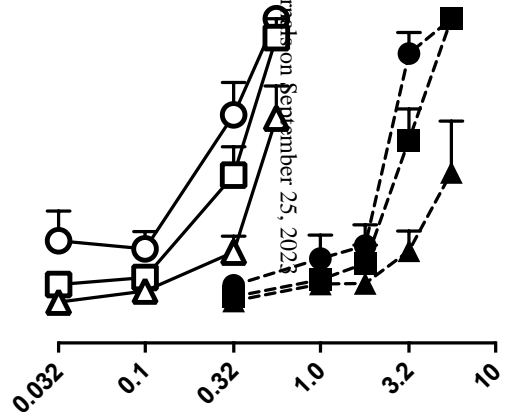
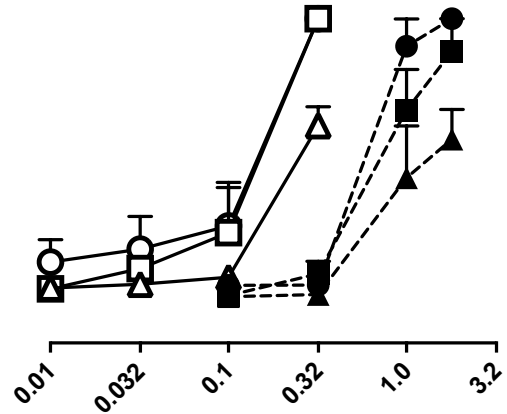
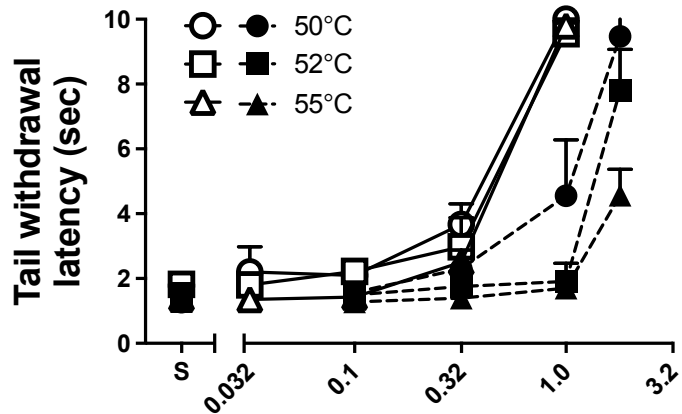


Figure 3 - Chronic naltrexone



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Figure 4 - Chronic naltrexone



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Figure 5 - Chronic naltrexone

