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## Punishment and reinforcement by opioid receptor agonists in a choice procedure in rats

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### Abstract

Intravenous (i.v.) drug self-administration remains the “gold standard” for assessing abuse potential of drugs. Failure of a drug to maintain self-administration might indicate merely the absence of positive reinforcing effects but might also indicate presence of aversive effects. Sensitivity to aversive effects is thought to affect the initiation and maintenance of drug use as well as relapse. Choice procedures are used to study positive reinforcing effects of drugs and to a much lesser extent to study punishing effects of drugs. Experiment 1 compared the *mu* opioid receptor agonist remifentanyl (0.001–0.01 mg/kg/infusion), the *kappa* receptor agonist spiradoline (0.0056–0.056 mg/kg/infusion), and histamine (1.0 mg/kg/infusion) in rats choosing between a food pellet only and an intravenous (i.v.) infusion + a food pellet. To test whether a history with one punishing drug affects the punishing effects of a second drug, Experiment 2 compared sensitivity to spiradoline in rats with and without a history of histamine punishment. All rats predominantly chose a pellet alone when histamine + a pellet was the alternative, and they predominantly chose remifentanyl + a pellet over a pellet alone. In Experiment 2, spiradoline was punishing in rats with a history of histamine punishment but not drug-naïve rats. This food choice procedure is sensitive to reinforcing and punishing effects of different drugs in the same subjects, suggesting that the procedure is well-suited for studying drug mixtures (e.g., *mu* and *kappa* agonists) and the impact of different physiological conditions (e.g., pain) on reinforcement and punishment.

### Keywords

opioid; aversion; reinforcement; punishment; choice; operant behavior; drug history; rat

### INTRODUCTION

Intravenous (i.v.) drug self-administration remains the gold standard for assessing abuse potential of drugs and candidate treatments for substance abuse (Hursh et al., 2005; Panlilio and Goldberg, 2007; Howell and Fantegrossi, 2009). For a broad range of drugs from different classes (with several noteworthy exceptions), there is a strong positive (predictive)

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relationship between positive reinforcing effects in nonhumans (i.e., self-administration) and the likelihood of abuse. Failure of a drug to maintain self-administration reflects the absence of positive reinforcing effects. However, most self-administration single- or concurrent schedule procedures, in which only positive reinforcing effects are measured, do not provide information on causal factors for the absence of drug-maintained responding. Other drug effects influencing self-administration might become apparent under conditions where both positive reinforcing and punishing effects of drugs can be studied in the same subjects.

The behavioral, including reinforcing, effects of drugs are impacted by a variety of factors, including drug and behavioral history as well as the particular environmental conditions under which they are studied. For example, squirrel monkeys have been shown to respond reliably on one lever to receive cocaine infusions and concurrently respond on a second lever to terminate scheduled cocaine infusions (Spealman, 1979). Monkeys self-administer i.v. nicotine alone but not nicotine paired with i.v. remifentanil when remifentanil alone is available (Koffarnus and Winger, 2015). These and other studies clearly demonstrate that all drugs have dose-dependent multiple effects suggesting that other (non-reinforcing or aversive) effects of a drug might contribute to or limit the expression of its reinforcing effects.

Choice procedures appear to be especially well suited for examining how the aversive effects of drugs might influence the extent to which a drug is self-administered and/or is likely to be abused (e.g., Riley, 2011; Verendeev and Riley, 2013). However, this approach has received little attention to date, with only a limited number of studies having been conducted to examine the potentially important aversive effects of drugs with reinforcing properties (Woolverton, 2003; Podlesnick et al., 2010; Woolverton et al., 2012; Podlesnick and Jimenez-Gomez 2013; Freeman et al., 2014; Koffarnus and Winger, 2015). A stimulus (e.g., drug) has punishing effects if response-dependent presentation of that stimulus decreases the frequency of responding (Azrin and Holz 1966; Skinner 1953); in choice procedures, a punishing effect is indicated by the re-allocation of behavior to an alternative response (e.g., lever) rather than the suppression of responding.

The present study used a food versus drug + food choice procedure in rats to compare opioid receptor agonists that act selectively at either *kappa* or *mu* receptors. Spiradoline (*kappa* agonist) was studied because it produces dysphoria in humans (Pfeiffer et al., 1986; Wadenberg, 2003) and conditioned place avoidance in rats (Rech et al., 2011), presumably reflecting its aversive properties. Remifentanil (*mu* agonist) was studied because it maintains responding in self-administration procedures under a broad range of conditions (e.g., Cooper et al., 2008; Sukhtankar et al., 2014), reflecting its positive reinforcing effects. Histamine also was studied because it has served as an effective punisher in choice procedures, presumably reflecting its aversive properties (Woolverton, 2003; Podlesnick et al., 2010; Woolverton et al., 2012; Podlesnick and Jimenez-Gomez 2013). It is well understood that behavioral or pharmacological history, as well as behavioral experiences during initial drug exposure, can determine subsequent drug effects (e.g., Hoffmeister and Schlichting, 1972; Smith et al., 1978; Stein et al., 2013, 2015). Thus, this study also tested whether exposure to one drug (i.e., spiradoline or histamine) alters the later exposure to a second drug (i.e., histamine or spiradoline, respectively).

## METHODS

### Subjects

Twenty-six male Sprague-Dawley rats (Envigo, Indianapolis, Indiana, USA) weighed 250–275 g upon arrival and were individually housed in 45 × 24 × 20 cm plastic cages with rodent bedding and free access to water. Rats had free access to standard rodent chow (Envigo Teklad) for one week while habituated to handling. Thereafter rats were food restricted to 15 ± 5 g after daily sessions, and allowed to grow to a maximum body weight of 375 ± 5 g. A 14:10 h light:dark cycle was in effect (lights on at 06.30 h) with sessions conducted during the light period.

Ten rats were in Experiment 1 (due to loss of catheter patency or illness, 4 did not complete the study). Prior to the start of Experiment 1, rats responded under fixed-ratio schedules for different pellet amounts (i.e., 3 versus 1 and 2 versus 1) and different volumes, rates, and durations of histamine infusions. These initial parametric studies were conducted in order to optimize the experimental design; the terminal parameters and criteria described below were selected based on findings from these preliminary studies.

Sixteen additional rats were experimentally-naïve at the start of Experiment 2 and were randomly assigned to two groups of 8 after recovery from surgery; all 16 rats completed Experiment 2. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio and in accordance with guidelines set forth by the Guide for the Care and Use of Laboratory Animals.

### Apparatus

Sessions were conducted in commercially available operant conditioning chambers (31 × 24 × 21 cm; ENV-008CT; Med Associates, Inc., St Albans, Vermont, USA) enclosed in ventilated, sound-attenuating cubicles (ENV-022M; Med Associates, Inc.). The side panels of the chamber were Plexiglas, and the rear and front panels were aluminum. The front panel was equipped with two response levers horizontally aligned 11.5 cm apart. Above each lever was a 2.5-cm diameter stimulus light that could be trans-illuminated white with a 100 mA bulb (lever lights). A feeder dispensed 45-mg grain-based pellets (F0165; Bio-Serv, Frenchtown, New Jersey, USA) to a 5 × 5 cm food aperture centrally located between the two levers. The rear panel was equipped with a 100 mA house light centered 2 cm from the top of the chamber. A syringe pump (PHM-100, Med Associates, Inc.) delivered i.v. infusions through Tygon tubing connected to a 22-G single channel plastic swivel (375/22PS; Instech Laboratories, Inc., Plymouth Meeting, Pennsylvania, USA) and spring tether (VAB95T; Instech Laboratories, Inc.) that attached to a vascular access button (VAB95BS; Instech Laboratories, Inc.) on the back of the rat. MED-PC IV software and a PC-compatible interface (Med Associates, Inc.) controlled experimental events and recorded data.

## Surgery

Rats were anesthetized with 2% isoflurane and prepared with a chronic indwelling catheter (CNC-3H-30-6/6.5; Access Technologies, Skokie, Illinois, USA) in the left femoral vein. The catheter was tunneled subcutaneously (s.c.) to the mid-scapular region, where it attached to a 22-G access port (VAB95BS; Instech Laboratories, Inc.) anchored in a s.c. pocket. Immediately after surgery, rats received s.c. injections of 0.5 ml penicillin (300,000 U/ml) and 1.0 mg/kg meloxicam and were allowed 7 days to recover before resuming sessions. Catheters were flushed with 0.5 ml of heparinized saline (100 U/ml) daily during recovery and after each session and flushed with 0.5 ml of sterile saline before each session. If catheter patency was compromised, as indicated by resistance in flushing, abnormal behavior, leakage of fluid, or failure to achieve sedation immediately after an infusion of 3.2 mg/kg of methohexital, another catheter was implanted in the right femoral vein or a jugular vein.

## Procedure

Sessions comprised up to 100 trials in a maximum of 100 min. The first two trials were sampling trials in which either the left lever or right lever was active, signaled by illumination of the left or right stimulus light, respectively. The position (i.e., left or right) was selected at random for the first sampling trial, and the alternative lever was presented in the second sampling trial. The remaining trials (up to 98) were choice trials in which both levers were active, signaled by the illumination of both stimulus lights. A single response to an active lever extinguished the stimulus lights, resulted in a brief (0.1-s) flash of the house light, and delivered a food pellet. Food delivery was followed by a 10-s inter-trial interval, during which the stimulus lights were extinguished and lever presses had no scheduled consequence. For all trials, failure to respond within a 60-s limited hold was scored as an omission and initiated the inter-trial interval. Rats had a minimum of 7 consecutive sessions in which both sampling trials and at least 90% of choice trials were completed before surgery.

The procedure following surgery was identical to that described above, except that responding on one lever delivered a food pellet while responding on the other lever delivered an i.v. infusion + a food pellet. The lever designation (i.e., left or right) associated with the infusion + a pellet was switched twice (A-B-A reversal design) for each drug and dose studied. Lever reversals occurred after a minimum of 3 sessions according to the following criteria: 1) percent choice for the infusion + a pellet decreased to 25% or less for histamine and spiradoline tests (based on demonstrated and hypothesized aversive effects, respectively); 2) percent choice for the infusion + a pellet increased to at least 75% for remifentanyl tests (based on demonstrated positive reinforcing effects); or 3) a maximum of 7 sessions. For saline tests, the lever reversals occurred after a minimum of 3 consecutive sessions in which both sampling trials and at least 90% of choice trials were completed. When drug or dose was changed, the infusion initially was paired with the preferred lever (i.e., the lever selected on more than 50% of choice trials) from the final session in the preceding condition; if a rat responded equally on both levers in the preceding condition the lever position was selected randomly.

**Experiment 1: Effects of histamine, spiradoline, and remifentanil on choice.—**

The purpose of this experiment was to use a choice procedure (Podlesnick et al. 2010) to test opioid receptor agonists for reinforcing and punishing effects in the same subjects. After testing saline, histamine (1.0 mg/kg/infusion) was tested to confirm sensitivity to a drug previously shown to be a punisher when paired with food in similar choice procedures (Woolverton 2003; Podlesnick et al. 2010; Podlesnick and Jimenez-Gomez 2013). Next, the *kappa* opioid receptor agonist spiradoline (0.056 and 0.0056 mg/kg/infusion) was tested. *Kappa* agonists produce dysphoria in humans and place avoidance conditioning as well as suppression of ongoing self-administration in nonhumans, presumably reflecting their aversive properties (Mucha and Herz 1985; Pfeiffer et al. 1986; Glick et al. 1995; Schenk et al. 1999; Wadenberg 2003). Spiradoline was tested to determine whether punishing effects were evident with a drug from a different pharmacological class (i.e., a *kappa* opioid receptor agonist compared with histamine used in previous studies). These doses of spiradoline were selected on the basis of our prior work with this compound in rats and its potency relative to the *kappa* agonist U50488 that has been studied extensively in rats (Di Chiara and Imperato 1988; Holtzman 2000; Morani et al. 2009). In contrast to *kappa* agonists, *mu* opioid receptor agonists are abused by humans and readily self-administered by nonhumans. Two doses of the *mu* opioid receptor agonist remifentanil (0.01 and 0.001 mg/kg/infusion) were tested to determine whether this procedure was sensitive to positive reinforcing effects in addition to punishing effects in the same subjects. These doses of remifentanil were selected on the basis of its potency as a positive reinforcer in other studies (O'Connor and Mead 2010; Panlilio and Schindler 2000). Histamine (1.0 mg/kg/infusion) was studied before and after remifentanil to test for possible changes in punishing effects that might result from experience with a drug having positive reinforcing effects.

**Experiment 2: Drug history and the punishing effects of spiradoline.—**

Because the order in which drugs were tested was the same for all subjects in Experiment 1, it was unclear whether exposure to histamine affected the subsequent exposure to spiradoline. To test this possibility, a 2-sequence crossover design was used in Experiment 2 to examine possible differences in punishing effects when histamine was tested before spiradoline and, in a separate group of rats, when spiradoline was tested before histamine. Saline was studied first in all rats (n=16). Next, one group (n=8) was tested with i.v. histamine (1.0 mg/kg/infusion) while a second group (n=8) was tested with i.v. spiradoline (0.056 mg/kg/infusion). If a rat completed an average of fewer than 20 choice trials for both lever designations (see lever switching as described above), then the dose was decreased to 0.32 mg/kg/infusion for histamine (1 rat) and 0.0056 mg/kg/infusion for spiradoline (6 rats) before testing the second drug in the sequence. When spiradoline was tested in rats that initially received histamine, 6 rats required additional testing with a smaller dose (0.0056 mg/kg/infusion); and when histamine was tested in rats that initially received spiradoline, 2 rats required additional testing with a smaller dose (0.32 mg/kg/infusion).

**Drugs**

Histamine dihydrochloride (Sigma-Aldrich, St. Louis, MO), spiradoline mesylate (Upjohn, Kalamazoo, Michigan, USA), and remifentanil hydrochloride (National Institute on Drug Abuse Drug Supply Program, Bethesda, Maryland, USA) were dissolved in sterile 0.9%

saline. I.V. infusions were in a volume of 1.0 ml/kg body weight at a rate of 3.36 ml/min. Methohexital sodium was generously provided by Eli Lilly and Company (Indianapolis, Indiana, USA), dissolved in sterile 0.9% saline, and administered in a volume of 1.0 ml/kg. All doses are expressed in terms of the salt.

## Data Analyses

For each session, the number of choices for a pellet only and the number of choices for the infusion + a pellet were divided by the total number of choice trials completed to obtain percent choice for each alternative. Percent choice for a pellet only was subtracted from percent choice for the infusion + a pellet as a measure of relative choice (i.e., difference scores), with positive values indicating preference for the infusion + a pellet, negative values indicating preference for a pellet alone, and values not different from 0 indicating no preference (i.e., indifference). To construct dose-response functions for relative choice and number of trials completed, the last session from each condition (A-B-A) was included to obtain a mean value for each rat. A one-way repeated measures ANOVA with Bonferroni's post-hoc test (Experiment 1) and a two-way ANOVA with Bonferroni's post-hoc test (Experiment 2) were used to analyze relative choice and the number of trials completed for each dose compared with saline; differences were considered significant for  $p < 0.05$ . Analyses were conducted using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

## RESULTS

### Experiment 1.

When choosing between a pellet only and saline + a pellet, rats were generally indifferent to saline infusions and responded approximately equally on the two levers, yielding a relative choice value that was not significantly different from zero (Fig 1, triangle, upper panel). When choosing between a pellet only and histamine + a pellet, rats chose predominantly a pellet only (Fig 1, circle, upper panel;  $t=7.9$ ,  $p < 0.001$ ). Neither saline nor histamine decreased the number of trials completed (Fig 1, lower panel). When choosing between a pellet and remifentanyl + a pellet, rats chose 0.01 mg/kg/infusion + a pellet over a pellet only (Fig 1, diamonds, upper panel;  $t=6.7$ ,  $p < 0.001$ ), despite a significant decrease in the number of trials completed (Fig 1, lower panel;  $t=4.2$ ,  $p < 0.002$ ). The effects on choice and trials completed with a 10-fold smaller dose of remifentanyl (0.001 mg/kg/infusion) were not different from saline. When choosing between a pellet and 0.056 mg/kg/infusion spiradoline + a pellet, rats responded predominantly for a pellet only (Fig 1, squares, upper panel;  $t=6.0$ ,  $p < 0.001$ ); this dose of spiradoline significantly decreased the number of trials completed (Fig 1, lower panel;  $t=3.4$ ,  $p < 0.02$ ). The effects on choice and trials completed with a 10-fold smaller dose of spiradoline (0.0056 mg/kg/infusion) were not different from saline. The effects of histamine in each of two re-determinations (data not shown) were not significantly different from the initial effects of histamine.

Fig 2 shows detailed patterns of responding across 3 consecutive sessions for a representative rat. The left panels show a summary measure (percent choice for the infusion + a pellet) for each session, while the right panels show a corresponding heat map depicting the response type for each of 100 trials. Omitted trials (no response within 60 sec) are

indicated by white; responding for a pellet only is indicated by light gray shading; and responding for an infusion + a pellet is indicated by dark gray shading. Percent choice for an infusion of saline + a pellet neither increased nor decreased systematically over 3 consecutive sessions (Fig 2, upper left panel), with responses for each alternative evenly distributed within each session (Fig 2, upper right panel). Percent choice for an infusion of 1.0 mg/kg/infusion histamine or 0.056 mg/kg/infusion spiradoline + a pellet decreased progressively across 3 consecutive sessions to less than 25% (Fig 2, center left panels). Responses for the infusion (histamine or spiradoline) + a pellet were evenly distributed at the start of a session and later in the session, with many trials being omitted (Fig 2, center right panels). By the third session with histamine or spiradoline, fewer trials were omitted as the rat increasingly chose the pellet only; responding for the infusion + a pellet tended to be concentrated towards the beginning of the session (Fig 2, center right panels). Percent choice for an infusion of 0.01 mg/kg/infusion remifentanyl + a pellet increased across 3 consecutive sessions to a maximum of greater than 90% (Fig 2, lower left panel). The relatively few choices for remifentanyl + a pellet in the first session were evenly distributed across trials (Fig 2, lower right panel). Responding for remifentanyl + a pellet increased in the second and third sessions (Fig 2, lower left panel) with responding for the pellet alone concentrated towards the start of the session (Fig 2, lower right panel).

## Experiment 2.

When choosing between a pellet only and saline + a pellet, all rats were generally indifferent to saline infusions, responding on the two levers approximately equally (Fig 3, upper panel). The rats that were tested first with histamine chose a pellet alone over histamine + a pellet (Fig 3, black circles, upper panel;  $t=5.0$ ,  $p<0.001$  for 1.0 mg/kg/infusion). Rats that received histamine first also chose predominantly a pellet alone when the alternative was spiradoline + a pellet (Fig 3, black squares, upper panel;  $t=3.2$  for 0.0056 and 0.056 mg/kg/infusion,  $p<0.01$ ). In contrast, rats that were tested first with spiradoline responded approximately equally on each lever with relative choice not being different from saline (Fig 3, gray squares, upper panel;  $t=0.51$  for 0.0056 mg/kg/infusion and  $t=0.75$  for 0.056 mg/kg/infusion, NS); however, rats tested first with spiradoline subsequently chose a pellet alone when the alternative was histamine + a pellet (Fig 3, gray circles, upper panel;  $t=2.9$ ,  $p<0.02$  for 0.32 mg/kg/infusions and  $t=4.2$ ,  $p<0.001$  for 1.0 mg/kg/infusion). In both groups of rats, spiradoline and histamine decreased the number of trials completed in a dose-related manner (Fig 3 lower panel).

## DISCUSSION

One purpose of the current study was to test the sensitivity of a food versus drug + food choice procedure to reinforcing and punishing effects of *mu* and *kappa* opioid receptor agonists, respectively. This study also compared the effects of spiradoline (*kappa* receptor agonist) in rats with and without histamine experience to test the hypothesis that exposure to one drug affects the response to subsequent exposure to second drug. Histamine had punishing effects in all rats, consistent with previous reports using food choice in rats (Podlesnick et al. 2010) and monkeys (Woolverton 2003) and drug choice in monkeys (Woolverton et al. 2012). Remifentanyl was reinforcing (i.e., rats chose predominantly a food

pellet that was paired with an infusion of remifentanyl over a food pellet alone) whereas spiradoline was punishing (i.e., rats chose predominantly a food pellet alone over a food pellet that was paired with an infusion of spiradoline) in rats that previously received histamine; however, spiradoline was not punishing in drug-naïve rats.

Overall, this choice procedure was sensitive to both reinforcing effects of remifentanyl (*mu*) and punishing effects of spiradoline (*kappa*) in the same subjects. The ability to study reinforcing and punishing effects in the same subjects offers an advantage over single- or concurrent-schedule procedures in which animals do or do not respond for drug. Under those conditions, the absence of self-administration is typically interpreted as merely the absence of positive reinforcing effects since those procedures are not sensitive to possible aversive effects of drugs. Hoffmeister and Wuttke (1973) distinguished between positive reinforcing and negative reinforcing effects of various opioids in monkeys by using an escape/avoidance protocol in which responses to a lever either terminated a drug infusion (escape) or postponed scheduled infusions (avoidance). Nalorphine and cyclazocine, which both have actions at *kappa* receptors, engendered high levels of escape and avoidance responding compared to saline, whereas the *mu* agonist codeine was well-tolerated, as evidenced by complete extinction of escape and avoidance responding. Note that the absence of responding is interpreted as a positive reinforcing effect, whereas the reallocation of responding among two or more alternatives is the primary measure in choice procedures. Place conditioning procedures can distinguish between rewarding and aversive effects; however, as each subject typically is exposed to only a single condition (Cunningham et al. 2006), adequately powered studies that include dose-response determinations studies for multiple drugs requires a very large number of subjects.

The relative choice for a pellet alone was approximately –80 when histamine + a pellet was the alternative and –65 when spiradoline + a pellet was the alternative. This difference might result from the criteria used for changing experimental conditions (i.e., changing conditions when choice for one alternative was greater than 75%); if the same condition was studied for more sessions, the effects of histamine and spiradoline might have been more similar. However, that spiradoline was punishing in rats that had received histamine but not in drug-naïve rats suggests that histamine is a more effective punishing stimulus, perhaps due to its very rapid onset and offset of action compared with spiradoline (e.g., Bovet et al. 1958; Chang et al. 2011). It will be important to understand how variations on onset and duration of action impact the reinforcing and punishing effects of drugs under procedures such as the one used in this study.

Spiradoline likely accumulated within a session, potentially impacting performance across trials: spiradoline might be a more effective punisher in drug-naïve rats if, for example, infusions were available less frequently (e.g., long inter-trial intervals). The onset of the aversive stimulus is critical for establishing a punishment effect with non-drug stimuli (e.g., shock). Delaying an aversive stimulus like shock or a histamine infusion, even by a few seconds, reduces the effectiveness substantially (Banks and Vogel-Sprott 1965; Woolverton et al. 2012). Experience with histamine appeared to be important in establishing a punishing effect for spiradoline, but it is not the case that experience with histamine resulted in punishing effects for all subsequently studied drugs. In Experiment 1, rats received



histamine before (and after) remifentanyl, which had robust positive reinforcing effects. The possibility also remains that longer acting *mu* agonists, such as morphine, might produce less robust preference as compared to remifentanyl.

In the current experiments, there were marked differences in drug effects between rats with different histories. In Experiment 1, 0.0056 mg/kg/infusion spiradoline + a pellet was without effect on choice or trials completed; however, this dose of spiradoline had significant effects on choice (punishing) in Experiment 2. Moreover, 1.0 mg/kg/infusion of histamine decreased the number of trials completed in Experiment 2 but not in Experiment 1. Spiradoline was an effective punisher in histamine-experienced but not drug-naïve rats. These difference in effectiveness of spiradoline as a punisher might be related to a global difference in sensitivity of rats in the two studies to punishment. Hoffmeister and Wuttke (1973) showed that monkeys readily responded to escape and avoid infusions of nalorphine and cyclazocine after extensive training with shock avoidance, although it is not known whether experience with shock avoidance was necessary to establish the negative reinforcing effects of nalorphine and cyclazocine. Likely, many factors affect punishing effects of drugs just as many factors affect positive reinforcing effects of drugs. For drug-maintained responding, subjects with an extensive history of reinforcement respond at higher rates and for lower doses of drug, compared with subjects that have a more limited history of drug taking (Goldberg 1973). Systematic investigation of a wide range of parameters and a variety of drugs will be necessary in order to understand the complex relationships among positive reinforcement, punishment, drug history, behavioral history, and environmental conditions.

One apparent advantage of this food versus drug + food choice procedure, compared with single- or concurrent-schedule procedures (Maguire et al., 2013; Weed et al., 2017), is that it can distinguish between reinforcing effects, punishing effects, and the general suppression of behavior. These features will be useful for studying interactions between reinforcing and punishing effects of drugs administered alone and in mixtures as well as any experimental manipulation (i.e., treatment) that might influence the relative contribution of each effect. Pain can modulate reinforcing and aversive effects of drugs (Shippenberg et al., 1988; Martin et al., 2006; Martin and Ewan, 2008; Li, 2013; Wade et al., 2013). One possibility is that titrating the doses of one drug that is a positive reinforcer and another drug that is a punisher might yield a mixture that effectively treats pain with little to no abuse potential (i.e., is indifferent in this choice procedure). The food choice procedure used in the present study likely would be appropriate for evaluating drug mixtures. Punishing effects of *kappa* agonists and reinforcing effects of *mu* agonists might be mutually antagonized in a drug mixture (e.g., *mu/kappa*) or by a physiological state (e.g., pain). If unwanted effects of each drug can be significantly reduced or avoided, then *mu/kappa* mixtures might be advantageous to *mu* opioids alone for treating pain.

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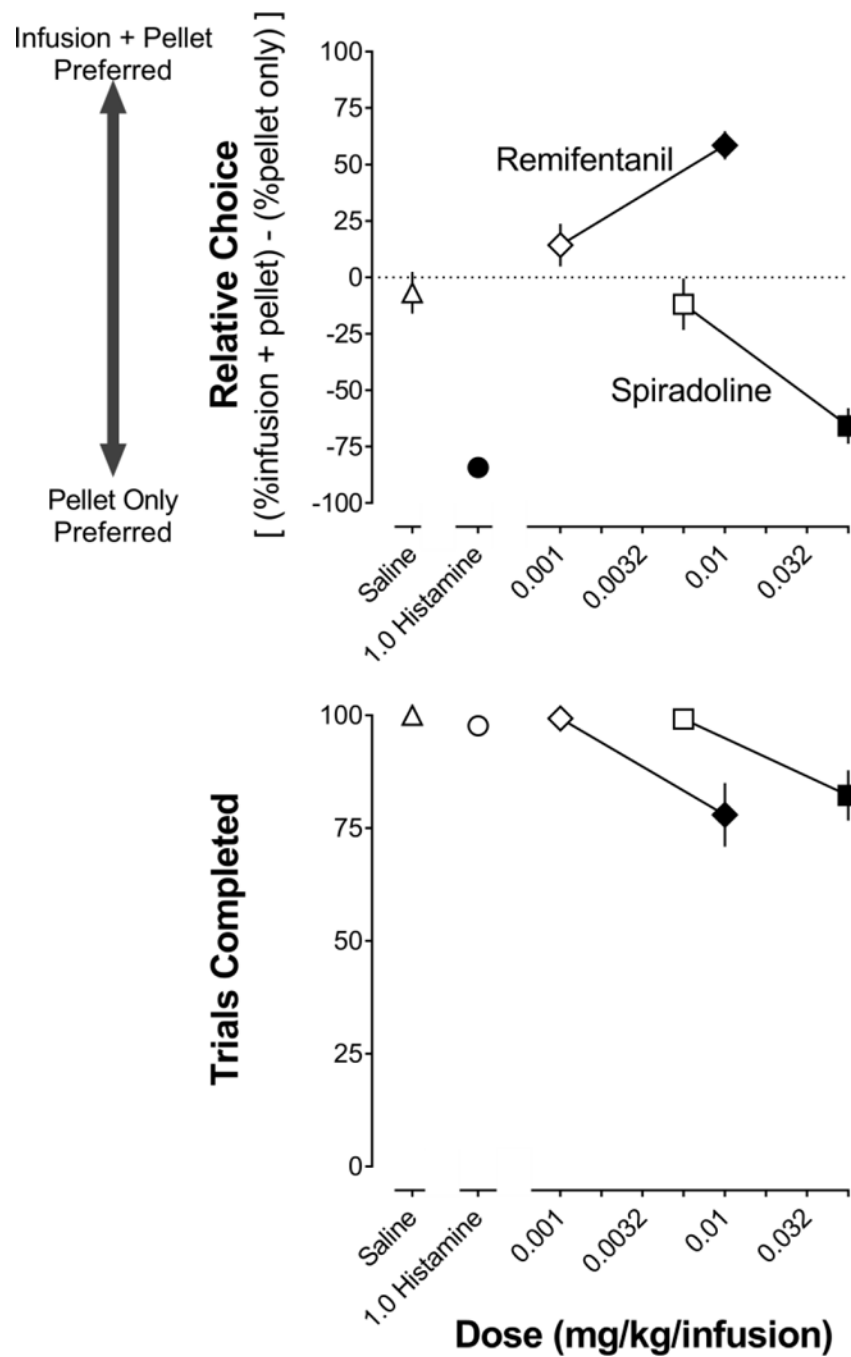
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**Figure 1.** Mean ( $\pm 1$  SEM) relative choice for each alternative (upper) and the number of trials completed (lower) during saline (triangles), histamine (circles), remifentanil (diamonds), and spiradoline (squares) tests in Experiment 1. Ten rats completed tests with saline and histamine, 7 rats completed tests with spiradoline (0.056 mg/kg/infusion), and 6 rats completed the remaining tests (spiradoline [0.0056 mg/kg/infusion], remifentanil [0.001–0.01 mg/kg/infusion], and histamine redetermination). Data shown are for only the 6 rats

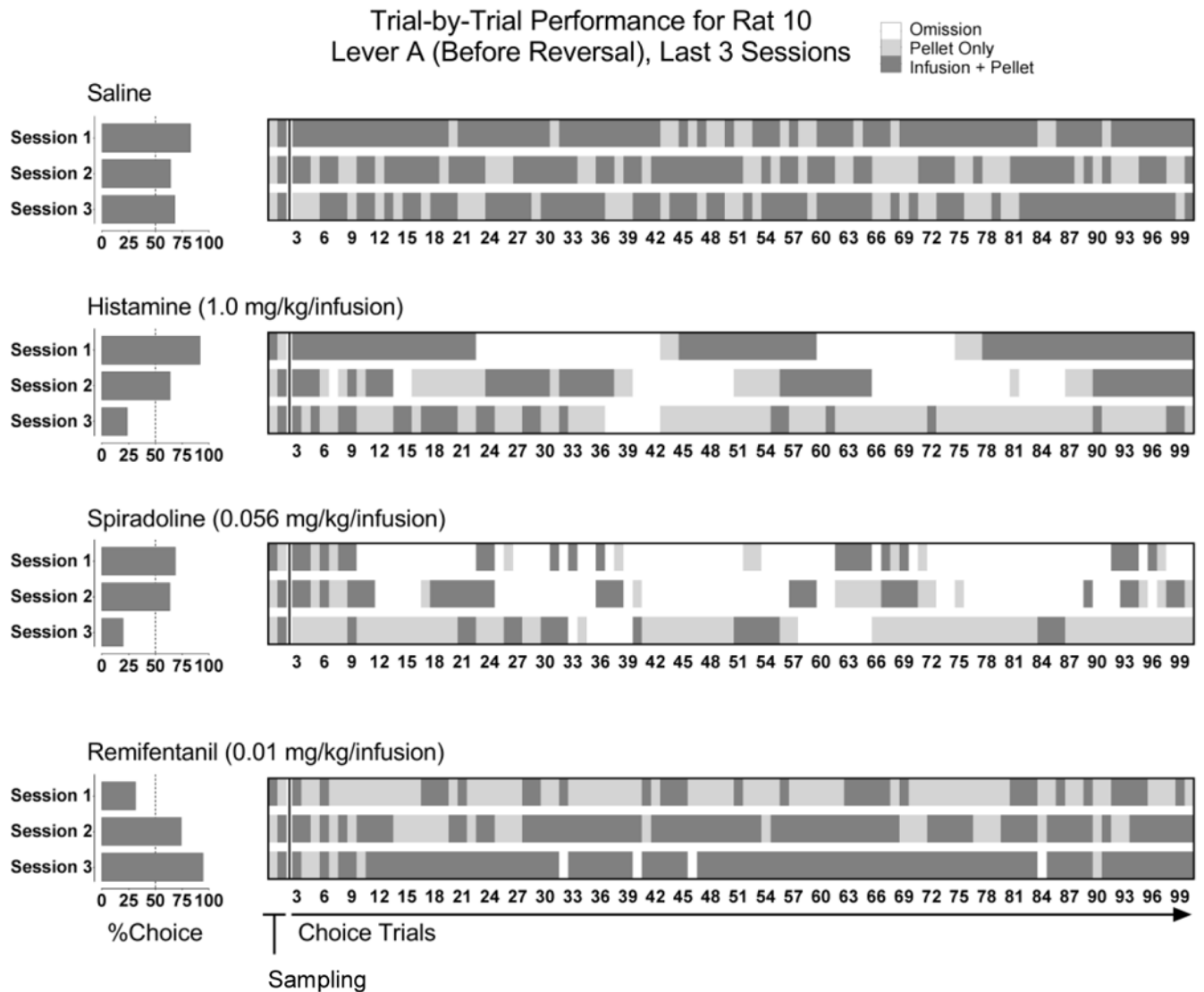
that completed the study, with filled symbols denoting a significant ( $p < 0.05$ ) effect compared with saline. C: unit dose in mg/kg/infusion.

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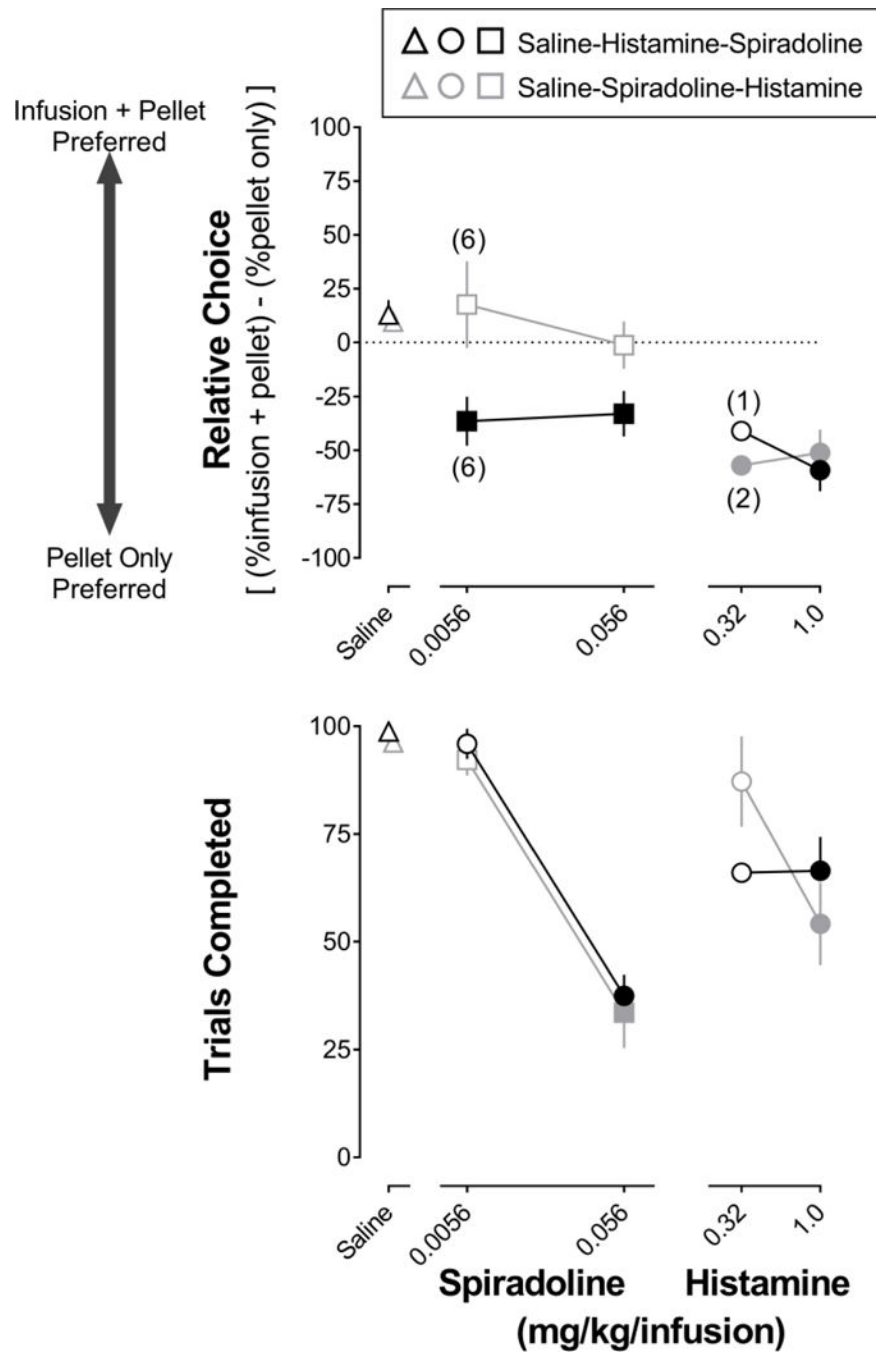
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**Figure 2.**

Representative patterns of responding for an individual rat across 3 consecutive sessions of saline, histamine, spiradoline, or remifentanil paired with one lever (Lever A; i.e., before a lever reversal). Percent choice for the infusion + a pellet (left panels) and corresponding heat maps depicting response type for each trial (right panels). Trial-by-trial performance is color-coded as follows: white represents no response (an omission); light gray represents a response for a pellet only; and dark gray represents a response for the infusion + a pellet. The preceding condition to saline was no infusion (i.e., before surgery). The preceding condition to histamine (1.0 mg/kg/infusion) was saline (paired with Lever A, reversal). The preceding condition to both spiradoline (0.056 mg/kg/infusion) and remifentanil (0.01 mg/kg/infusion) was histamine (paired with Lever A, reversal).



**Figure 3.** Mean ( $\pm$  SEM) relative choice for each alternative (upper) and the number of trials completed (lower) during saline (triangles), histamine (circles), and spiradoline (squares) tests in Experiment 2. Black outlined symbols represent 8 rats that were tested with histamine prior to spiradoline. Gray outlined symbols represent 8 rats that were tested with spiradoline prior to histamine. Filled symbols denote a significant ( $p < 0.05$ ) effect compared



with saline. Each data point represents 8 rats, except where noted by numbers in parentheses. Horizontal axes: unit dose in mg/kg/infusion.

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