Title: Long-lasting effects of methocinnamox on opioid self-administration in rhesus monkeys

Running title: Methocinnamox and opioid self-administration

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Abstract

Opioid abuse remains a serious public health challenge, despite the availability of medications that are effective in some patients (naltrexone, buprenorphine, and methadone). This study explored the potential of a pseudoirreversible *mu* opioid receptor antagonist (methocinnamox; MCAM) as a treatment for opioid abuse by examining its capacity to attenuate the reinforcing effects of *mu* opioid receptor agonists in rhesus monkeys. In one experiment, monkeys responded for heroin (n=5) or cocaine (n=4) under a fixed-ratio schedule. Another group (n=3) worked under a choice procedure with one alternative delivering food and the other alternative delivering the *mu* opioid receptor agonist remifentanil. A third group (n=4) responded for food and physiological parameters were measured via telemetry. Effects of MCAM were determined in all experiments and, in some cases, were compared to those of naltrexone. When given immediately before sessions, naltrexone dose-dependently decreased responding for heroin and decreased choice of remifentanil while increasing choice of food, with responding returning to baseline levels one day after naltrexone injection. MCAM also decreased responding for heroin and decreased choice of remifentanil while increasing choice for food; however, opioid-maintained responding remained decreased for several days following treatment. Doses of MCAM that significantly decreased opioid-maintained responding did not decrease responding for cocaine or food. MCAM did not impact heart rate, blood pressure, body temperature, or activity at doses that decreased opioid self-administration. Because MCAM can selectively attenuate opioid self-administration for prolonged periods, this novel drug could be a safe and effective alternative to currently available treatments for opioid abuse.

Introduction

Opioid abuse remains a serious public health challenge that is responsible for substantial morbidity, mortality, and social costs (Stotts et al. 2009; Birnbaum et al. 2011). In 2016, 11.8 million people in the United States reported misusing an opioid, including heroin and prescription opioid pain relievers, and 2.1 million individuals met the criteria for an opioid use disorder (SAMHSA 2017). Despite high rates of abuse, only 15 to 20% of individuals receive any type of treatment (Becker et al. 2008; Saloner and Karthikeyan 2015), and for individuals that receive treatment, there is a high risk for relapse (e.g., Lee et al. 2018). Thus, there is an unmet clinical need to expand and improve treatment of individuals with opioid use disorders.

Medication-assisted therapy is considered the first line treatment for opioid use disorder and currently approved medications include methadone, buprenorphine, and naltrexone (Schuckit 2016). Methadone and buprenorphine have agonist actions at *mu* opioid receptors, share many effects with abused opioids such as heroin, and serve as replacements for abused opioids. However, while effective in many patients, both drugs have limitations. Methadone is only available through outpatient treatment programs, where it is dispensed daily; the frequency of dosing can be quite burdensome and is not feasible for many patients. Buprenorphine has a relatively long duration of action, and recently developed extended release formulations (e.g., Probuphine®) increase this duration significantly. However, only specialists can prescribe buprenorphine and implants require minor surgical procedures for insertion and removal, limiting the number of health care providers that are available and/or willing to prescribe this drug. Because of agonist effects, treatment with methadone and buprenorphine can result in physical dependence and carries a risk for diversion and overdose. Moreover, agonists at *mu* opioid receptors interact with other drugs such as alcohol and benzodiazepines to produce potentially

dangerous drug-drug interactions (e.g., Hall et al. 2008; Jones et al. 2012; Jones et al. 2014; Jones and McAninch 2015).

Naltrexone (Revia®) is an opioid receptor antagonist that blocks the effects of opioids. Although naltrexone avoids abuse liability and adverse effects associated with methadone and buprenorphine, its pharmacological properties limit its effectiveness. Naltrexone has a relatively short duration of action and binds to opioid receptors in a competitive, reversible manner; therefore, its antagonist effects can be surmounted by administering large doses of an agonist. An injectable extended-release formulation of naltrexone (Vivitrol®) increases its duration of action significantly but does not mitigate the fact that its antagonist actions are surmountable. Moreover, administration of an opioid antagonist can precipitate withdrawal in opioid-dependent individuals; thus, naltrexone is used in patients following a period of detoxification from opioids. In summary, although currently available medications are effective for opioid abuse in some patients, there is a significant need for more and better approaches to treat this public health emergency (Volkow and Collins 2017).

Methocinnamox (MCAM) is an opioid receptor antagonist, developed as a pharmacological tool that selectively eliminates *mu* opioid receptors. MCAM attenuates binding of the selective *mu* opioid receptor ligand [^{3}H]DAMGO in washed membranes obtained from mice, suggesting that MCAM binds in a functionally (pseudo)irreversible manner (Broadbear et al. 2000). Irreversible drugs such as β-funaltrexamine bind covalently to receptors (e.g., Portoghese et al. 1980); however, drugs such as clocinnamox, buprenorphine, and MCAM are thought to be pseudoirreversible insofar as they dissociate very slowly from receptors but do not form a covalent bond. MCAM was shown to be an effective antagonist of *mu* opioid receptor-mediated effects *in vivo*. For example, MCAM attenuated the antinociceptive effects of morphine

in mice and rats (Broadbear et al. 2000; Peckham et al. 2005) and was superior to irreversible (e.g., β-funaltrexamine) and pseudoirreversible (e.g., clocinnamox) *mu* opioid receptor antagonists with respect to potency and selectivity at *mu* opioid receptors (Broadbear et al. 2000). Previous studies have demonstrated the effectiveness of irreversible and pseudoirreversible *mu* opioid receptor antagonists for blocking opioid self-administration in nonhuman subjects (Negus et al. 1993; Martin et al. 1995; Martin et al. 1998; Zernig et al. 1997). However, the impact of MCAM on abuse-related effects of opioids, including self-administration, has not been characterized.

This study explores the therapeutic potential of MCAM for treating opioid abuse by examining its capacity to attenuate opioid self-administration in rhesus monkeys. In one experiment, iv infusions of heroin or cocaine were delivered according to a fixed-ratio schedule of reinforcement. Effects of MCAM were compared to those of naltrexone, which is the only antagonist approved for treating opioid abuse. Effects on cocaine self-administration were determined to characterize the selectivity of MCAM for attenuating opioid-maintained behavior. In a second experiment, monkeys could choose between food and iv infusions of the *mu* opioid receptor agonist remifentanil. This experiment extends the evaluation of MCAM to include another opioid receptor agonist, the ultra-potent and short-acting fentanyl analogue remifentanil. Under choice procedures, changes in reinforcing effects are characterized based on allocation of behavior among alternatives, rather than overall response output that can be sensitive to many factors other than reinforcing effectiveness. In a third experiment, responding for food and physiological parameters (heart rate, blood pressure, temperature, and activity) were monitored to characterize direct effects of MCAM, including its effects on responding maintained by a non-drug reinforcer.

Materials and Methods

Animals. Thirteen adult rhesus monkeys (10 males [AC, AP, DU, FI, GI, LO, MA, MO, TI, and WI] and 3 females [NI, PR, and RU]), weighing between 6.2 and 14.6 kg, were housed individually in stainless steel cages in colony rooms maintained under a 14/10-h light/dark cycle (lights on at 0600 h). Monkeys were fed chow (Harlan Teklad, High Protein Monkey Diet, Madison, WI, USA), fresh fruit, peanuts, and other treats daily to maintain body weights, and water was available continuously. Studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the United States National Institutes of Health (National Research Council, 2011), and were approved by the University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee.

Surgery. Monkeys were sedated with 10 mg/kg (im) of ketamine (Henry Schein Animal Health, Dublin, OH, USA), intubated, and then maintained on 2 l/min oxygen and isoflurane anesthesia (Butler Animal Health Supply, Grand Prairie, TX, USA). Penicillin B&G (40,000 IU/kg) and meloxicam (0.1-0.2 mg/kg) were given postoperatively. During surgery and under anesthesia, an intravenous (iv) catheter or a telemetry transmitter was implanted. For monkeys in the heroin self-administration study, a 5-french polyurethane catheter (Access Technologies, Skokie, IL, USA) was inserted into a jugular, femoral, or subclavian vein, tunneled sc to the mid-scapular region of the back, and attached to a sc vascular access port (MIDA-PU-C50, Access Technologies). For monkeys in the choice study, a 5-french polyurethane catheter was inserted into a vein, tunneled to the mid-scapular region of the back, passed through a flexible stainless steel tether (Lomir Biomedical, Quebec, Canada), and connected to an 18-g single-channel fluid swivel (Lomir Biomedical) that was secured to the rear wall of the home cage. The opposite side

of the swivel, located outside of the cage, was attached to a 30-ml syringe mounted in a syringe pump (Razel Scientific Instruments, Fairfax, VT, USA). Monkeys wore a jacket (Lomir Biomedical) that protected the catheter and secured the tether. For monkeys in the study in which responding was maintained by food, a telemetry transmitter (PhysioTel Digital Implant model L11, Data Science International, St. Paul, MN) was placed in the left or right flank, with positive ECG leads tunneled to the lower-left quadrant of the thorax, negative ECG leads tunneled to the upper-right quadrant, and the systemic blood pressure catheter tunneled to and inserted into the left or right femoral artery. During the course of the study, monkeys in the choice and food-maintained responding studies were sedated every 2 weeks with 10 mg/kg of ketamine to obtain an updated body weight, confirm catheter patency (choice study only), perform routine health checks, and inspect the equipment.

Heroin and cocaine self-administration. Five monkeys (AC, LO, NI, PR, and RU) participated in the heroin self-administration experiment and 4 monkeys (AC, FI, LO, and PR) participated in the cocaine self-administration experiment; all had experience lever-pressing for iv infusions of drug, including heroin and/or cocaine. Three monkeys (AC, LO, and PR) participated in both experiments.

Daily sessions were conducted seven days per week. Monkeys were seated in primate chairs (Model R001, Primate Products, Miami, FL, USA) and positioned in sound-attenuating operant-conditioning chambers containing two horizontally aligned response levers located approximately 32 cm apart. Above each lever was a circular, translucent disk that could be trans-illuminated green or red. Self-administered drug infusions were delivered iv by connecting a 185-cm extension set (Abbott Laboratories, Stone Mountain, GA, USA) to the vascular access port by a 20-g Huber-point needle (Access Technologies). The distal end of the extension set was

connected to a 30- or 60-ml syringe mounted in a syringe pump (PHM-100, Med Associates) located outside the chamber. White noise and an exhaust fan masked extraneous sounds. Experimental events were arranged and data were recorded through an interface (Med Associates) connected to a PC computer operating Med-PC IV software (Med Associates).

Prior to the start of the session, the port and catheter were flushed with 5 ml of a sterile 0.9% saline solution and a syringe and extension set containing the solution available for self-administration that day was connected to the Huber-point needle and mounted in the syringe pump. One minute before the start of the session, the pump was activated to load the catheter with the new solution. Sessions began with noncontingent delivery of a priming infusion; a red light over the active lever was illuminated for 5 sec and the dose of drug available for self-administration during that session was delivered. Immediately after the priming infusion, the light above the active lever (side counterbalanced across monkeys) was illuminated green signaling the beginning of the response period. Thirty consecutive responses (fixed-ratio [FR] 30 schedule) on the active lever changed the light from green to red for 5 sec and delivered an iv infusion. Completion of the response requirement also initiated a 180-sec timeout, during which all lights were off (except for the red light that was illuminated for 5 sec) and responses were recorded but had no programmed consequence. Following the timeout, the light was again illuminated green, signaling the next response period. Responses on the other (inactive) lever during response periods reset the response counter for the active lever and had no other programmed consequence. Lever designations remained constant for each monkey for the duration of the study. Sessions lasted for 90 min, inclusive of response and timeout periods. At the end of the session, the catheter and port were flushed and locked with 3 ml of heparinized saline (100 U/ml) to maintain catheter patency.

In some sessions, monkeys could respond for heroin (0.0032 mg/kg/infusion), and in other sessions they could respond for cocaine (0.032 mg/kg/infusion). Occasionally, monkeys received a sc injection prior to the session (described below). Four types of tests were conducted as follows: 1) naltrexone pretreatment with heroin self-administration; 2) MCAM pretreatment with heroin self-administration; 3) naltrexone pretreatment with cocaine self-administration; and 4) MCAM pretreatment with cocaine self-administration. Pretreatment injections were given 15 or 60 min prior to the session for tests with naltrexone and MCAM, respectively. Each test comprised multiple sessions, with saline injections given sc 15 or 60 min before the session, depending on the test, for at least one session prior to and following each test injection; during tests with MCAM, saline injections were given 60 min prior to the session for 3 sessions following the test injection.

Effects of naltrexone and MCAM on heroin self-administration were determined in 5 monkeys (AC, LO, NI, PR, and RU). A naltrexone dose-effect curve was determined 3 times during the experiment. Each determination began once responding for heroin was stable, as defined by 3 consecutive sessions in which the average number of infusions obtained in each of those sessions varied by not more than ± 20% of the 3-session mean. Doses of naltrexone (0.0032, 0.01, and 0.032 mg/kg) were tested in an irregular order; on one occasion 0.1 mg/kg of naltrexone was tested in one monkey to complete the dose-response curve in that subject. After the first dose of naltrexone was tested, additional doses were tested when the number of infusions obtained during a single session was ≥ 80% of the initial 3-day baseline. For the first naltrexone dose-effect curve determination, each dose was tested twice, and effects of each dose were averaged. For subsequent naltrexone determinations (see below), each dose was only tested once. Effects of MCAM (0.032 and 0.32 mg/kg) on heroin self-administration were

determined in the same five monkeys once responding for heroin was stable according to the 3-day criterion described above; tests with MCAM were separated by at least 3 weeks and a re-determination of the naltrexone dose-effect curve to confirm that the potency of naltrexone was not different from the initial determination. Because the duration of effect of MCAM differed across monkeys, repeated tested with naltrexone within individual subjects provided evidence for each individual that the baseline status of the opioid system had been restored between consecutive tests with MCAM. Effects of naltrexone and MCAM on cocaine self-administration were determined in 4 monkeys (AC, FI, LO, and PR) once responding for cocaine was stable according to the 3-day criterion described above. Naltrexone (0.032 mg/kg) was tested first followed at least 3 days later by MCAM (0.32 mg/kg). Heroin and cocaine were dissolved in 0.9% sterile saline and delivered iv in a volume of 1 ml per 10 kg of body weight. Naltrexone and MCAM were dissolved in sterile water and delivered sc in the lower part of the back in a volume of 1.0-2.1 ml per 10 kg of body weight. Multiple injections were given when the total volume exceeded 0.8 ml.

Remifentanil versus food choice. Three monkeys (GI, MO, and WI) participated in the choice experiment; all had experience lever pressing for food and iv infusions (e.g., Maguire and France 2018). For these monkeys, the home cage (81 cm tall by 81 cm wide by 72 cm deep) also served as the experimental chamber. A stainless steel instrument panel (20 cm high by 28 cm wide) was mounted on one wall of the cage. The panel contained 2 levers (4.5 cm wide) horizontally aligned 23 cm above the floor and spaced 15 cm apart center to center. Three stimulus lights were horizontally aligned 5 cm above the levers. A 6 cm high by 5 cm wide aperture was located directly above the instrument panel through which 300-mg raspberry

flavored sucrose pellets (5TUT, Test Diet, Richmond, IN, USA) were delivered via activation of a pellet dispenser (Med Associates, Inc., Fairfax, VT, USA).

Daily sessions were conducted seven days per week and comprised four blocks. Each block began with 2 sample trials, followed by 10 choice trials. At the beginning of a sample trial, the light above one lever was illuminated green, and 30 consecutive responses on the lever directly below that light turned the side light off, turned on the white center light for 2 sec, initiated a 180-sec timeout, and delivered the reinforcer (one 300-mg sucrose pellet or an iv infusion) associated with that lever for the current block. Responses during the timeout had no programmed consequence. After the timeout, the other side light was illuminated green and 30 responses on the lever directly below that light delivered the associated reinforcer followed by a timeout. The order of sample trials varied randomly across blocks, and both sample trials had to be completed before choice trials were presented. During choice trials, both side lights were illuminated green and 30 consecutive responses on either lever delivered the reinforcer associated with that lever. For all trials, responses on one lever reset the response requirement for the other lever. The block ended after 45 min or completion of 12 trials (2 sample trials plus 10 choice trials), whichever occurred first. Blocks were separated by a 5-min blackout. If all trials were completed before the block ended, then the blackout was extended to include the remaining time in the block plus the blackout time. The catheter was flushed and locked with 3 ml of heparinized saline (100 U/ml) at the end of each session to maintain catheter patency.

During some sessions, the dose of remifentanil available increased in half-log units across blocks from 0.032 to 1.0 μg/kg/infusion. Occasionally, saline was substituted for remifentanil in all the blocks (saline-only session) to ensure that responding was sensitive to the availability of remifentanil rather than time in the session. Food was always available for

responding on one lever and an infusion was always available for responding on the other lever; lever designations (e.g., food, left; infusion, right) remained constant for individual monkeys for the duration of the experiment.

Tests were conducted once remifentanil dose-effect curves were stable, as indicated by 3 consecutive sessions in which more than 36 choice trials (90%) were completed and the percentage choice of drug across all blocks of the session did not vary by more than 20%. Because many choice patterns can produce identical values of percentage of drug choice for the entire session, visual inspection of the data also confirmed that the remifentanil dose-effect curves increased in a monotonic fashion. Three types of tests were conducted as follows: 1) naltrexone pretreatment given immediately before a remifentanil choice session; 2) naltrexone pretreatment given 24 hr prior to a remifentanil choice session; and 3) MCAM given 24 hr prior to a remifentanil choice session. In order to ensure that 24 hr intervened between injection of an antagonist and the availability of remifentanil during a choice session, MCAM and naltrexone (24 hr pretreatment) were given immediately prior to a saline-only session. Remifentanil was available during the next session and for each session thereafter. Tests with MCAM were separated by at least three weeks. All pretreatments were delivered iv through the catheter line (followed by a saline flush) immediately prior to the start of a session. Remifentanil, naltrexone, and the smallest dose of MCAM tested in this experiment (0.32 mg/kg) were dissolved in 0.9% sterile saline and delivered in a volume of 1 ml per 10 kg of body weight. The two larger doses of MCAM (1.0 and 3.2 mg/kg) were dissolved in a solution of saline containing 10% w/v β-cyclodextrin and injected in volumes of 1.0-3.2 ml per 10 kg of body weight. Comparable volumes of saline or β-cyclodextrin vehicle alone were administered prior to control sessions.

Food-maintained responding and physiological effects. Four monkeys (AP, DU, MA, and TI) participated in this experiment; all had experience lever pressing for food. For these monkeys, behavioral sessions and acquisition of telemetry data were conducted in their home cages, which were identical to those described for the choice experiment, although these monkeys did not wear a jacket or tether.

Behavioral sessions were conducted daily, seven days per week, and comprised five 10-min blocks that began at 30-min intervals. The beginning of a block was signaled by illumination of the green light above the right lever. When the light was on, 10 responses on the right lever delivered one 300-mg raspberry flavored sucrose pellet and turned off the green light for 0.5 sec. Responses on the left lever reset the response requirement on the right lever; blocks lasted for up to 10 food presentations or 10 min, which ever occurred first. All stimulus lights were turned off between blocks and responding during that time had no programmed consequence. Telemetry data were acquired using the Ponemah Software System (version 5.2) and associated hardware (Data Science International); measures included arterial blood pressure (mmHg), heart rate (beats per minute), body temperature (°C), and activity (counts).

Tests were conducted once food-maintained responding was stable, as indicated by 3 consecutive sessions in which the average response rate for each of those 3 sessions (aggregated across all 5 blocks of the session) did not deviate from the overall mean of those 3 sessions by more than 20%. A vehicle injection was administered 1 hr prior to the session on the first day of the test, and MCAM was administered 1 hr prior to the session on the following day. Sessions were conducted thereafter but no further injections were administered. Telemetry data were collected every minute for 48 h, beginning with the vehicle injection on the first day of the test. The smaller dose of MCAM (0.32 mg/kg) was tested first in four monkeys. Because of

a limited supply of drug, the larger dose of MCAM (3.2 mg/kg) was tested later in only 2 monkeys (DU and MA); injections of MCAM were separated by 33 days. For the first test (0.32 mg/kg), sessions began at 1000 h, and for the second test (3.2 mg/kg), sessions began at 1200 hr. Monkeys were given their daily food ration approximately 2 hr after the session ended. MCAM was dissolved in 10% w/v β-cyclodextrin vehicle and injected sc in the lower back in volumes of 1.0 to 3.2 ml per 10 kg of body weight. Multiple injections were given when the total volume exceeded 0.8 ml; a comparable number of injections of vehicle alone were administered on day 1 of each test.

Drugs. Heroin (3,6-Diacetylmorphine) hydrochloride, naltrexone hydrochloride, remifentanil hydrochloride, and cocaine hydrochloride were generously provided by the National Institute on Drug Abuse Drug Supply Program (Rockville, MD, USA). MCAM was synthesized as previously described (Broadbear et al., 2000). Doses were calculated using the salt form. 2-Hydroxypropyl-β-cyclodextrin was purchased (Accela ChemBio, Inc., San Diego, CA, USA) and dissolved in 0.9% sterile saline at a concentration of 0.1 g per ml.

Data and statistical analyses. For the heroin and cocaine self-administration experiments, the primary dependent measure was the number of infusions obtained during each session. Effects of naltrexone and MCAM on heroin self-administration were analyzed using a two-way, repeated-measures ANOVA with session and dose as within-subject factors. Tests with naltrexone comprised 3 consecutive sessions, beginning with the session immediately preceding naltrexone administration and ending with the day after naltrexone. Tests with MCAM comprised at least 10 sessions, beginning with the session immediately preceding MCAM and ending at least 8 sessions after MCAM. Due to variation in the duration of effect of MCAM across individual subjects, only data from the first 8 days after MCAM administration were included in

the analysis. Effects of naltrexone and MCAM on cocaine self-administration were analyzed using a one-way, repeated-measures ANOVA with session as the factor. Post-hoc analyses were conducted using Dunnett's test with data collected after administration of an antagonist being compared to the baseline session. The potency of naltrexone for decreasing heroin self-administration was quantified by plotting the number of infusions obtained as a function of the log-transformed (base 10) dose of naltrexone. Dose-effect data for individual subjects were then fit with a straight line using linear regression, and the slope and the y-intercept were used to estimate the dose of naltrexone that decreased the number of infusions obtained by 50% (ED_{50}). The ED_{50} of naltrexone was analyzed across 3 determinations using a one-way, repeated-measures ANOVA with determination as a factor.

For the food/drug choice experiment, the primary dependent measures were the percentage of drug choice and total number of choice trials completed. Percentage of drug choice was calculated for each block by dividing the number of choices of drug (i.e., ratios completed on the drug lever during choice trials) by the total number of choices (e.g., ratios completed on either lever during choice trials) and multiplying by 100; percentage of drug choice for the entire session was calculated the same way except that the number of choices of drug and total choices were aggregated across blocks. Percentage of drug choice and trials completed were plotted as a function of remifentanil dose. Whenever possible, remifentanil dose-effect curves for individual subjects were fit with a straight line using linear regression and only data on the linear portion of the dose-effect curve. The slope and the y-intercept were used to estimate the dose of remifentanil that produced 50% drug choice (ED_{50}). Shifts in the remifentanil dose-effect curve following pretreatment with an antagonist were quantified by calculating a potency ratio, which was the ED_{50} following a test divided by the control ED_{50}.

Control dose-effect curves were generated using data from the most recent remifentanil choice session conducted before a test. Effects of MCAM on percentage of drug choice and trials completed for each session were analyzed using a two-way, repeated-measures ANOVA with session and dose of MCAM as within-subject factors.

For the experiment on food-maintained responding and telemetry, rate of responding for each block was calculated by dividing the total number of responses on the right lever while the green light was illuminated by the total amount of time that the green light was illuminated. There were no systematic differences across blocks; therefore, rate for the entire session was aggregated by taking the average across blocks. Because the number of subjects differed between tests (4 for 0.32 mg/kg and 2 for 3.2 mg/kg), effects of each dose of MCAM on food-maintained responding were analyzed separately using a one-way, repeated-measures ANOVA with session as the factor. Data used for the analysis were taken from 6 consecutive sessions, beginning with the session immediately preceding MCAM administration (i.e., after vehicle administration) and including the 4 days after MCAM administration. Data obtained via telemetry were collected every minute and then averaged for each hour for 48 h, beginning with the vehicle injection on the first day of the test. Telemetry data were analyzed separately for each dose of MCAM using a two-way, repeated-measures ANOVA with treatment (vehicle or MCAM) and hour as within-subject factors. All of the data collected between the vehicle injection and those data were compared on an hourly basis to the corresponding time-point following MCAM injection the following day. For significant treatment-by-hour interactions, a Dunnett's post-hoc test was used to compare data for each hour obtained following vehicle administration on the first day to data obtained during the corresponding hour the next day following MCAM administration.

Dose-effect curves were fit using Microsoft Excel 2016 (Redmond, WA, USA) and data analysis was conducted using NCSS version 10.0.13 (Kaysville, UT, USA).

Results

Heroin and cocaine self-administration. When 0.0032 mg/kg/infusion of heroin was available for self-administration, the group average number of infusions obtained during sessions that immediately preceded administration of an antagonist ranged from 22.8 to 25.4 (Figures 1a and 1b, data above B). Naltrexone dose-dependently decreased infusions obtained on the day of treatment (Figure 1a, data above T) with responding returning to baseline levels the next day (Figure 1a, data above 1); these data represent effects of naltrexone from the first determination of the dose-effect curve. For naltrexone, there was a significant main effect of session ($F[2,44]=37.45$, $p<.001$), a significant main effect of dose ($F[2,44]=5.37$, $p=.01$), and a significant session by dose interaction ($F[4,44]=7.28$, $p<.001$). Infusions obtained on the day of treatment with 0.01 and 0.032 mg/kg of naltrexone were significantly different from baseline (filled symbols). The mean (± 95% confidence interval) ED_{50} value for the first naltrexone determination was 0.011 (0.007-0.016) mg/kg, which was not significantly different from the second [0.009 (0.002-0033) mg/kg] or third [0.011 (0.005-0.018) mg/kg] determinations according to one-way ANOVA ($p=.93$). MCAM also dose-dependently decreased infusions of heroin obtained (Figure 1b). There was a significant main effect of session ($F[9,97]=2.99$, $p=.004$), a significant main effect of dose ($F[1,97]=55.3$, $p<.001$), and a significant session by dose interaction ($F[9,97]=2.63$, $p=.01$). Infusions obtained on the day of treatment with 0.32 mg/kg of MCAM and the first 5 days thereafter were significantly different from baseline. When 0.032 mg/kg/infusion of cocaine was available for self-administration, the group average number of infusions obtained during sessions that immediately preceded administration of an antagonist

ranged from 26.0 and 26.8 infusions (Figures 1c and 1d, data above B). Neither naltrexone (0.032 mg/kg; Figure 1c) nor MCAM (0.32 mg/kg; Figure 1d) significantly altered the number of cocaine infusions obtained (p=.49 and p=.88, respectively).

Figure 2 shows effects of 0.32 mg/kg of MCAM on heroin self-administration in individual monkeys. Responding for heroin was disrupted for an average (± 1 standard deviation) of 9.6 ± 5.7 sessions before returning to stable levels near baseline (according to the 3-session criterion), though there was some variability across monkeys. For monkeys AC and NI, MCAM reduced the number of infusions obtained for 18 and 13 sessions, respectively, after MCAM injection (Figures 2a and 2b). For monkeys RU and LO, the number of heroin infusions obtained was below baseline levels for 7 and 5 sessions, respectively, after MCAM injection (Figures 2c and 2d). For monkey PR, responding reached stability 8 days after treatment, but effects were less consistent across sessions; nevertheless, the number of infusions obtained was below baseline for 4 out of the first 6 sessions after MCAM injection (Figure 2e).

Remifentanil versus food choice. In monkeys responding under the food/drug choice procedure, choice of remifentanil increased (Figure 3a, circles) and choice of food decreased (Figure 3c, circles) across blocks as the dose of remifentanil increased, with doses of 0.32 and 1.0 μg/kg/infusion maintaining at least 80% drug choice (Figure 3b). The mean (± 95% confidence interval) ED_{50} value of the control remifentanil dose-effect curve for percentage of drug choice was 0.18 μg/kg/infusion. Injection of 0.032 mg/kg of naltrexone immediately before a remifentanil versus food choice session decreased choice of remifentanil and increased choice of food, shifting the remifentanil dose-effect curve rightward, on average, 3.1 (3.0-3.2) fold (inverted triangles), and not altering the number of trials completed (Figure 3d). The remifentanil dose-effect curve returned to control the next day (upright triangles). On a separate occasion,

the same dose of naltrexone was administered 24 hr before a remifentanil choice session (i.e., without an intervening remifentanil session) and it did not alter the remifentanil dose-effect curve for percentage of drug choice, although the number of choice trials completed decreased in some blocks (squares).

MCAM significantly attenuated percentage choice of remifentanil, shifting the remifentanil dose-effect curve rightward and downward in a dose-dependent manner (Figure 4, top row), and in most cases without altering the number of trials completed (Figure 4, bottom row). For example, 0.32 mg/kg of MCAM decreased choice of 0.32 μg/kg/infusion of remifentanil from 100 to 0% and choice of the larger dose of remifentanil from 100 to 50% one day after injection of MCAM (Figure 4a, upright triangles). The two larger doses of MCAM (1.0 and 3.2 mg/kg) reduced choice of all doses of remifentanil to 0% one day after treatment (Figures 4b and 4c). The time to recovery of the remifentanil dose-effect curve was also related to the dose of MCAM, with the curve fully recovering by 4 days after 0.32 mg/kg of MCAM (squares), by 8 days after 1.0 mg/kg (diamonds), and by 12 days after 3.2 mg/kg (X symbol).

Figure 5 depicts the percentage of drug choice and choice trials completed for a session before and for 8 sessions following injection of different doses of MCAM. Under control conditions, overall percentage of drug choice was between 50 and 60% on average (Figure 5a, data points above C), and all choice trials were completed each session (Figure 5b). For percentage of drug choice, there was a significant main effect of dose of MCAM ($F[2,79]=23.22$, $p<.001$), a significant main effect of session ($F[8,79]=14.23$, $p<.001$), but no significant dose by session interaction ($p=.37$) (Figure 5a). There was a significant main effect of dose of MCAM on the number of choice trials completed ($F[2,79]=4.11$, $p=.02$) but no main effect of session ($p=.74$) and no dose by session interaction ($p=.83$) (Figure 5b).

Food-maintained responding and physiological effects. Monkeys responded at an average rate of 1.5 responses per second and obtained the maximum number (50) of pellets during baseline sessions (Figures 6a and 6b, data points above B). MCAM administered 1 h prior to the session did not significantly impact rate of responding or the number of pellets earned on the day of treatment (data points above T) or for the 4 sessions that followed [0.32 mg/kg: rate (p=.09), pellets (p=.45); 3.2 mg/kg: rate (p=.19), pellets (p value not determined)].

During the two 24-h test periods following vehicle administration, mean blood pressure ranged from 83.1 to 115.1 mmHg (Figures 7a and 7b), mean heart rate ranged from 70.4 to 141.0 beats per minute (Figures 7c and 7d), mean body temperature ranged from 35.8 to 37.8 °C (Figures 7e and 7f), and mean activity counts ranged from 306 to 3189 (Figures 7g and 7h). All four measures tended to decrease during the dark cycle of the day, indicated by the shaded regions. For the test with 0.32 mg/kg of MCAM (Figure 7, left column), there was a significant main effect of hour on blood pressure ($F[23,191]=4.63$, $p<.001$), heart rate ($F[23,191]=6.98$, $p<.001$), body temperature ($F[23,191]=23.65$, $p<.001$), and activity ($F[23,191]=7.99$, $p<.001$) but no main effect of treatment or hour-by-treatment interaction for any of these measures ($p >.05$ for all dependent variables). For the test with 3.2 mg/kg of MCAM (right column), there was a significant main effect of hour on blood pressure ($F[23,95]=3.08$, $p<.001$), heart rate ($F[23,95]=4.00$, $p<.001$), body temperature ($F[23,95]=27.18$, $p<.001$), and activity ($F[23,95]=2.61$, $p=.002$). There was no main effect of treatment for any of these measures ($p >.05$ for all dependent variables); however, there were significant hour-by-treatment interactions for heart rate ($F[23,95]=2.24$, $p=.01$; Figure 7d) and activity ($F[23,95]=2.80$, $p=.001$; Figure 7h). The Dunnett's post-hoc test indicated that heart rate and activity 20 hr following administration of 3.2 mg/kg was significantly lower compared with heart rate during the same time period of the

previous day following administration of vehicle (filled squares); there was no significant difference at any other time point.

Discussion

Opioid abuse continues to be a significant public health problem. Currently available treatments (methadone, buprenorphine, and naltrexone) are effective in many patients. However, burdensome dosing requirements, limited access to adequate healthcare, surmountability of opioid receptor blockade (naltrexone), and potentially dangerous drug-drug interactions (methadone and buprenorphine) limit the effectiveness of these medications. Thus, there is a significant need for more and better approaches to treat opioid abuse. This study evaluated the opioid receptor antagonist MCAM for treating opioid abuse by examining its capacity to attenuate opioid self-administration in rhesus monkeys and comparing its effects to those of naltrexone, which is the only antagonist approved for treating opioid abuse.

One group of monkeys responded for iv infusions of heroin during daily sessions under a single-lever, fixed-ratio schedule of reinforcement. In that group, heroin maintained high, reliable rates of intake at a unit dose (0.0032 mg/kg/infusion) that is at or near the peak of the self-administration dose-effect curve in rhesus monkeys (Gerak et al. 2009; Li et al. 2012; Maguire et al. 2013). Another group of monkeys chose between sucrose pellets and iv infusions of the mu opioid receptor agonist remifentanil. Monkeys chose food over small doses of remifentanil and increasingly chose remifentanil as the unit dose increased across blocks within the session. The potency of remifentanil was comparable to its potency in previous studies (e.g., Maguire and France 2018), and control dose-effect curves were very stable across the study.

In both groups of monkeys that self-administered an opioid, naltrexone decreased opioid-maintained behavior as indicated by reduced heroin intake (Figure 1) and reduced choice of remifentanil (Figure 3). In the choice experiment, reduced responding for remifentanil was accompanied by increased choice of food. Like other opioid receptor antagonists, naltrexone promoted a reallocation of behavior away from responding for an opioid and toward responding for the non-drug alternative (food; e.g., Negus 2006). Effective doses of naltrexone in the current study were within the range of those that attenuate many effects of *mu* opioid receptor agonists in rhesus monkeys (e.g., France et al. 1990; Bowen et al. 2002; Gerak et al. 2003; Gerak and France 2007; Li et al. 2008; Maguire and France 2016) including self-administration (e.g., Rowlett et al., 1998). Despite the effectiveness of naltrexone, reductions in opioid intake were relatively short-lived. Naltrexone produced significant effects on the day of treatment; however, in both experiments responding returned to baseline levels the next day. The short duration of action of naltrexone in rhesus monkeys is consistent with previous studies showing that the potency of naltrexone begins to diminish as soon as 2 hours after injection (e.g., Gerak and France 2007).

MCAM also decreased opioid-maintained responding, and effects lasted much longer than those of naltrexone. In the heroin self-administration experiment, a single injection of 0.32 mg/kg of MCAM markedly reduced heroin intake on the day of treatment and for several days thereafter, with reductions lasting approximately 10 days on average. In the choice experiment, MCAM reduced responding for remifentanil and increased responding for food. The time to return to baseline responding appeared to be positively related to the dose of MCAM. For example, responding recovered completely 4 days after injection of 0.32 mg/kg of MCAM, whereas the remifentanil dose-effect curve continued to be shifted rightward and downward 4

days after injection of 3.2 mg/kg (Figures 4 and 5). For both experiments, there was variability among subjects in the duration and magnitude of the decreases in opioid intake; however, there was clear evidence in all subjects of a prolonged effect of MCAM exceeding that of naltrexone. Individuals in all experiments were tested with MCAM multiple times. In the heroin self-administration experiment, sensitivity to naltrexone was confirmed before a subsequent test with MCAM, increasing confidence that sensitivity of *mu* opioid receptors had recovered.

In a previous study, clocinnamox, a congener of MCAM and pseudoirreversible antagonist at opioid receptors, significantly attenuated self-administration of the *mu* opioid receptor agonists alfentanil and nalbuphine in rhesus monkeys, resulting in a flattening of the self-administration dose-effect curve that in some cases lasted for several days (Zernig et al. 1997). Although the antagonist effects of clocinnamox and MCAM are long lasting, MCAM is more potent and has greater selectivity for *mu* over *delta* and *kappa* opioid receptors (Broadbear et al. 2000); thus, it would be expected to have fewer complications than clocinnamox with regard to actions at other opioid receptors.

Collectively, these data provide evidence that MCAM produces significant and prolonged suppression of opioid self-administration in non-human primates and suggests that MCAM is superior to naltrexone in its duration of action. Given that adverse outcomes associated with abuse of many drugs, including opioids, often reflect disproportionate allocation of behavior to drug seeking and taking (e.g., Lamb and Ginsburg 2018), results from the choice experiment are particularly encouraging as far as a single injection of MCAM shifted responding toward the non-drug (food) alternative for several days.

The therapeutic utility of candidate medications for treating drug abuse depends upon whether the medication selectively reduces behavior related to abuse and not all other behavior.

Results of the choice experiment provide some evidence of selectivity as far as MCAM decreased responding for remifentanil but not responding for food. However, additional studies were conducted to explore further the selectivity of MCAM. In one experiment, monkeys could self-administer 0.032 mg/kg/infusion of cocaine, a dose near the peak of the cocaine self-administration dose-effect curve in monkeys (e.g., Collins et al. 2016). Doses of naltrexone and MCAM that significantly decreased heroin self-administration (0.032 and 0.32 mg/kg, respectively) did not alter responding maintained by cocaine on the day of treatment or for several days thereafter. These data are consistent with previous studies showing that naltrexone, as well as other opioid receptor antagonists, produce small and/or inconsistent effects on cocaine self-administration in rhesus monkeys (Mello et al. 1990; Winger et al. 1992; Mello et al. 1993; Rowlett et al. 1998). This selectivity has also been demonstrated using the *mu* opioid receptor alkylating drug β-funaltrexamine in rats self-administering heroin or cocaine (Martin et al. 1998). Similarly, naltrexone has been shown to block subjective effects (i.e., ratings on a visual analog scale of “How high are you?”) of the *mu* opioid receptor agonist hydromorphone but not of cocaine in humans (Walsh et al. 1996). MCAM had no effects on responding for food in a third group of monkeys, up to a dose of 3.2 mg/kg of MCAM, 10-fold larger than the dose that decreased opioid-maintained responding. Moreover, MCAM failed to markedly alter heart rate, blood pressure, body temperature, or general activity. There was a significant interaction between treatment and hour for heart rate and activity following administration of 3.2 mg/kg of MCAM, but a post-hoc analysis showed that the difference occurred at only one time point, 20 hr following MCAM administration. The failure of naltrexone and MCAM to alter responding for cocaine and for food or other physiological measures, along with the potency of naltrexone, is consistent with effects of naltrexone and MCAM being

mediated by antagonism at *mu* opioid receptors rather than “off-target” effects and, together with data from the choice experiment, rules out generalized response rate suppression.

Conclusions. MCAM, an analogue of buprenorphine with no efficacy at *mu* opioid receptors, dissociates very slowly from the receptor which results in effects comparable to those of an irreversible antagonist (Broadbear et al. 2000). Because of the very slow dissociation, MCAM has a long duration of antagonist action owing to significant depletion of the pool of functional *mu* opioid receptors. Presumably, the duration of effect is directly related to the number of receptors bound by MCAM and rate of turnover of *mu* opioid receptors (Zernig et al. 1994). Such a long duration of antagonist action might be preferable in some patients for treating opioid abuse especially when access to adequate healthcare is limited. Pseudoirreversible binding of MCAM to *mu* opioid receptors suggests that antagonist effects would not be surmounted by taking larger doses of an opioid agonist. Moreover, MCAM would significantly increase compliance with treatment in so far as binding of MCAM to opioid receptors cannot easily be reversed, for example, by removing an implant. Because MCAM has no efficacy at *mu* receptors (Broadbear et al. 2000), there is very low risk for development of physical dependence, and adverse pharmacodynamic interactions with other non-opioid drugs such as alcohol and benzodiazepines are not likely to occur. Thus, treatment with MCAM might be preferred for individuals that co-abuse opioids with alcohol or benzodiazepines. MCAM also reverses and prevents heroin-induced respiratory depression in monkeys at the same doses and for comparable periods, supporting the use of MCAM for treatment of opioid overdose (Gerak, unpublished observation). Because MCAM is an antagonist, it might also be expected to precipitate withdrawal in opioid-dependent individuals as do naloxone and naltrexone, the only opioid antagonists currently approved for treating opioid overdose and abuse, respectively.

Thus, like naltrexone, MCAM treatment might require a period of opioid detoxification before treatment induction.

MCAM decreased responding maintained by the opioid receptor agonists heroin and remifentanil but did not significantly decrease responding maintained by cocaine or food. Effects of MCAM lasted much longer than those of naltrexone, with a single injection of MCAM reducing opioid intake for several days or weeks. Formulations could be developed to deliver small doses of MCAM over a long period of time, which would provide continuous blockade of *mu* receptors. Doses of MCAM that significantly reduced opioid intake did not alter physiological measures such as blood pressure, heart rate, body temperature, and general activity, indicating a favorable safety profile of MCAM with regard to these physiological parameters. Taken together, these data demonstrate that MCAM can safely and effectively attenuate opioid self-administration for prolonged periods and suggest that this novel drug could be superior to currently available treatments for opioid abuse by enhancing compliance and increasing availability to patients with limited or no access to health care providers.

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Conducted experiments: Maguire

Contributed new reagents or analytic tools: Husbands, Disney

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Footnotes

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Figure legends

Figure 1. Effects of naltrexone and MCAM on self-administration of heroin or cocaine in rhesus monkeys. The mean (± 1 SEM) number of infusions of 0.0032 mg/kg/infusion of heroin (panels a and b; n=5) or 0.032 mg/kg/infusion of cocaine (panels c and d; n=4) is plotted across consecutive sessions. Data points above B indicate the number infusions obtained the session preceding administration of an antagonist. Data points above T indicate the number of infusions obtained during the session immediately following administration of an antagonist; the pretreatment time for naltrexone (panels a and c) was 15 min, and the pretreatment time for MCAM (panels b and d) was 60 min. The numbers along the abscissa show the time in days since the injection of an antagonist. Filled symbols indicate data that are significantly different from baseline according to a Dunnett's post-hoc test ($p<.05$).

Figure 2. Effects of 0.32 mg/kg of MCAM on self-administration of 0.0032 mg/kg/infusion of heroin for individual monkeys. Data are the same as plotted in Figure 1 and other details are the same as in Figure 1.

Figure 3. Effects of 0.032 mg/kg of naltrexone administered iv on choice between food and remifentanil in rhesus monkeys (n=3). Choices of remifentanil (panel a), choices of food (panel c), the percentage choice of remifentanil (panel b), and total number choice trials completed (panel d) are plotted as a function of unit dose of remifentanil (µg/kg/infusion). The dose of remifentanil increased across blocks of the session. Symbols indicate the mean (± 1 SEM) for the group. Circles indicate control data for dose-effect curves for remifentanil alone. Inverted

triangles indicate data from a session in which naltrexone was administered immediately prior to the session; upright triangles indicate data from the session that immediately followed. Squares indicate data from a session in which naltrexone was administered 24 h earlier, prior to a saline-only session (i.e., without an intervening remifentanil choice session).

Figure 4. Effects of a range of doses of MCAM administered iv on choice between food and remifentanil in rhesus monkeys (n=3). Data for tests with 0.32, 1.0, and 3.2 mg/kg of MCAM are shown in the first (panels a and d), second (panels b and e), and third (panels c and f) columns, respectively. MCAM was administered immediately prior to a saline-only session, followed by consecutive daily sessions in which remifentanil was available. Other details are the same as in Figure 3.

Figure 5. Summary data depicting the effects of MCAM administered iv on percentage of remifentanil choice and total choice trials completed for each session following administration of 0.32 (diamonds), 1.0 (triangles), and 3.2 (squares) mg/kg of MCAM (n=3). Data points indicate the mean (± 1 SEM) for the group.

Figure 6. Effects of MCAM administered sc on response rate and number of pellets obtained in monkeys responding under a fixed-ratio schedule of food delivery. Sessions were divided into 5 blocks that started every 30 min; data are collapsed across all blocks of the session.

Mean (± 1 SEM) response rate and pellets earned for the group are plotted across consecutive sessions. Data points above B are from the session preceding administration of an MCAM. Data points above T indicate data from the session beginning 60 min after MCAM administration. The numbers along the abscissa show the time in days since the injection of MCAM. Diamonds indicate data from the test with 0.32 mg/kg of MCAM (n=4), and squares indicate data from the test with 3.2 m/kg MCAM (n=2).

Figure 7. Effects of MCAM administered sc on physiological parameters in monkeys implanted with telemetry devices. Measures of arterial blood pressure (panels a and b), heart rate (panels c and d), body temperature (panels e and f), and activity (panels g and h) were collected every minute beginning with the vehicle injection on the first day of each two-day test and averaged each hour. Circles (all panels) indicate the group mean (± 1 SEM) for each hour for 24 h following vehicle administration. Diamonds (left column) and squares (right column) indicate data collected after administration of 0.32 and 3.2 mg/kg of MCAM, respectively. For the test with 0.32 mg/kg of MCAM, injections were given at 0900 h, and for the test with 3.2 mg/kg of MCAM injections were given at 1100 h. In both cases, injections were given 1 h prior to the start of the food-maintained operant behavior session. The shaded region indicates time when the lights were off in the colony room. Filled symbols indicate data obtained following MCAM administration that were significantly different from the corresponding hour following vehicle administration the previous day according to a Dunnett's post-hoc test ($p<.05$).

Figure 1

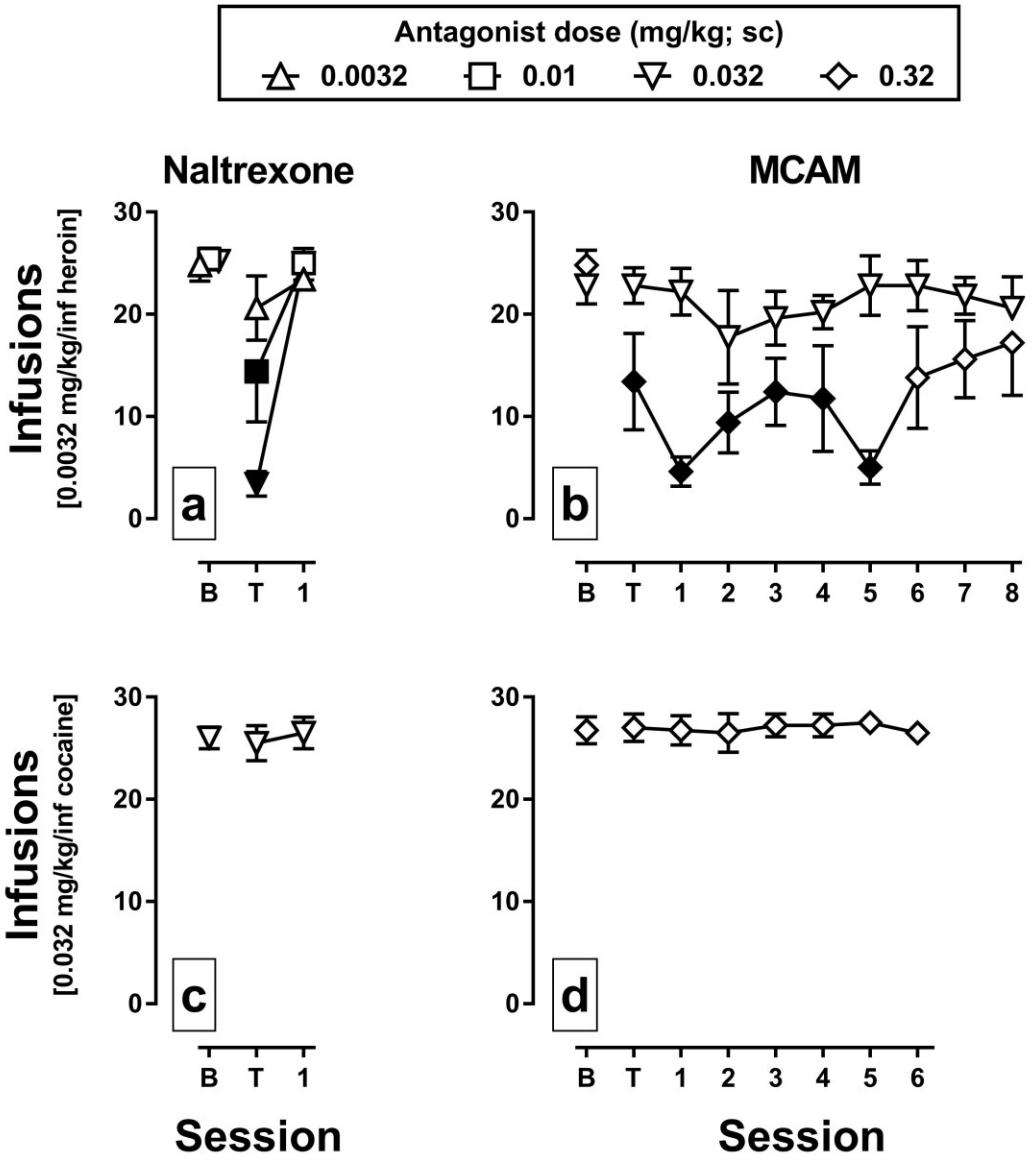

Antagonist dose (mg/kg; sc)
0.0032
0.01
0.032
0.32
Naltrexone
MCAM
Infusions
[0.0032 mg/kg/inf heroin]
Infusions
[0.032 mg/kg/inf cocaine]
a
b
c
d
B T 1
B T 1 2 3 4 5 6 7 8
B T 1 2 3 4 5 6
0
10
20
30
Session

Figure 2

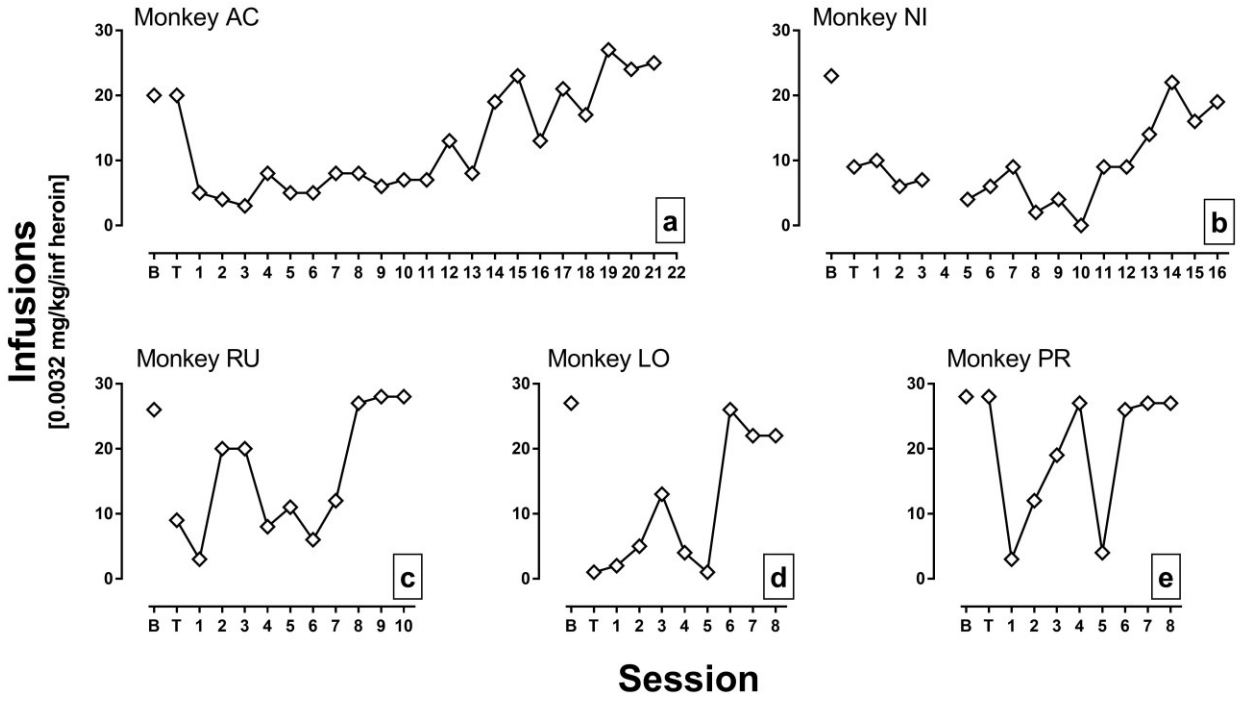

Monkey AC
Monkey NI
Monkey RU
Monkey LO
Monkey PR
Infusions
[0.0032 mg/kg/inf heroin]
Session
a
b
c
d
e

Figure 3

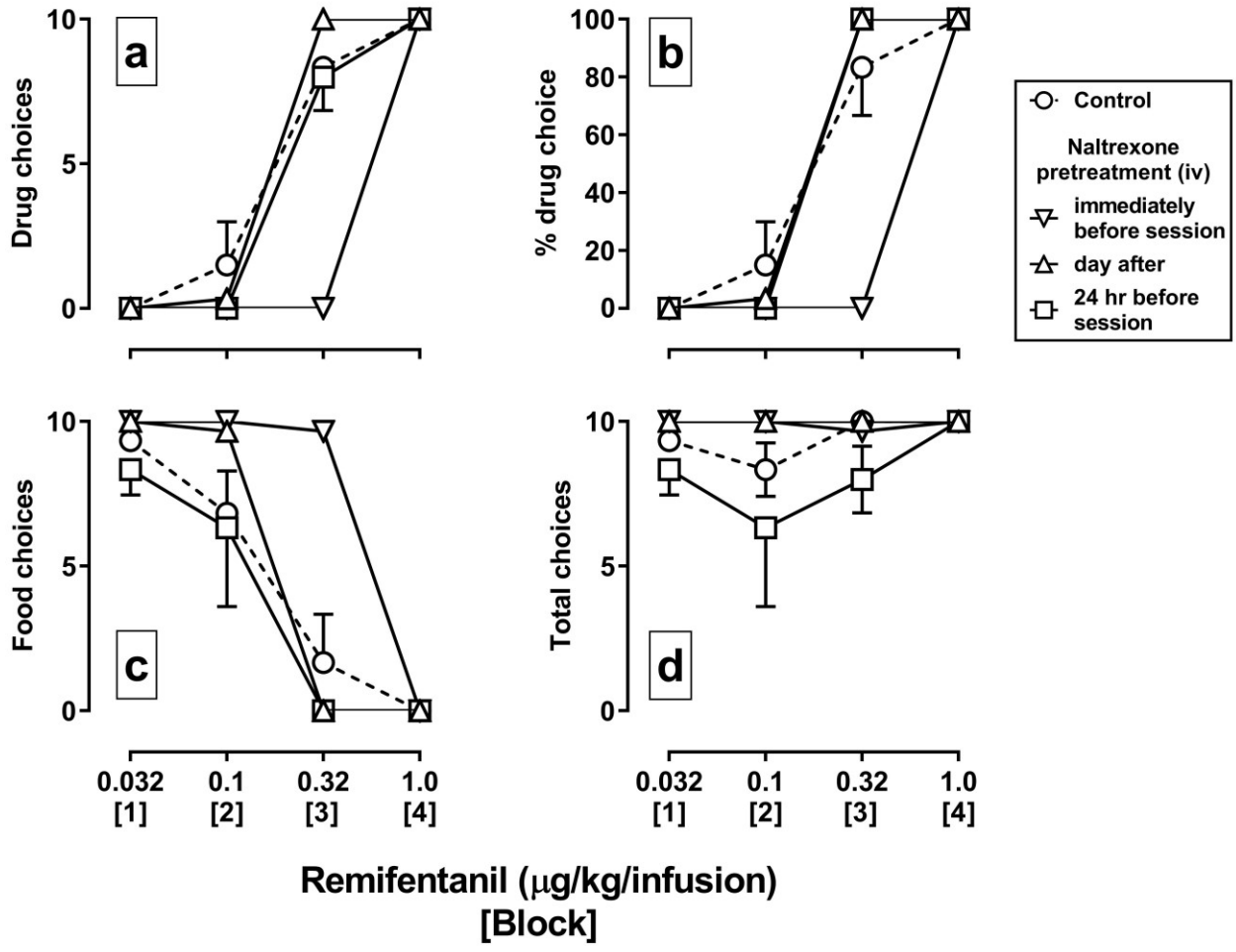

a
b
c
d
Drug choices
% drug choice
Food choices
Total choices
10
5
0
100
80
60
40
20
0.032
[1]
0.1
[2]
0.32
[3]
1.0
[4]
Control
Naltrexone pretreatment (iv)
immediately before session
day after
24 hr before session
Remifentanil (μg/kg/infusion)
[Block]

Figure 4

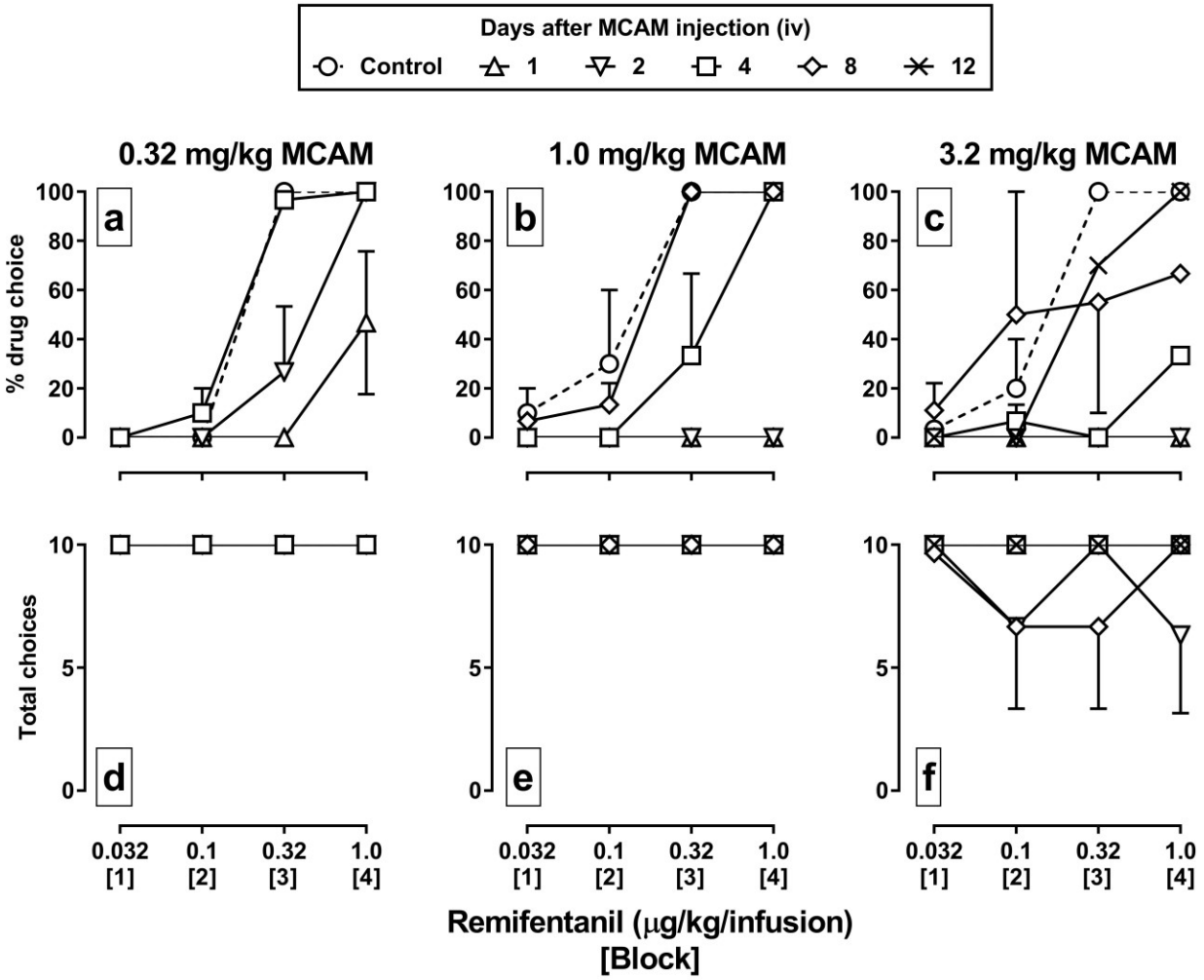

Days after MCAM injection (iv)
Control
1
2
4
8
12
0.32 mg/kg MCAM
1.0 mg/kg MCAM
3.2 mg/kg MCAM
a
b
c
d
e
f
% drug choice
Total choices
0.032 [1]
0.1 [2]
0.32 [3]
1.0 [4]
Remifentanil (μg/kg/infusion) [Block]

Figure 5

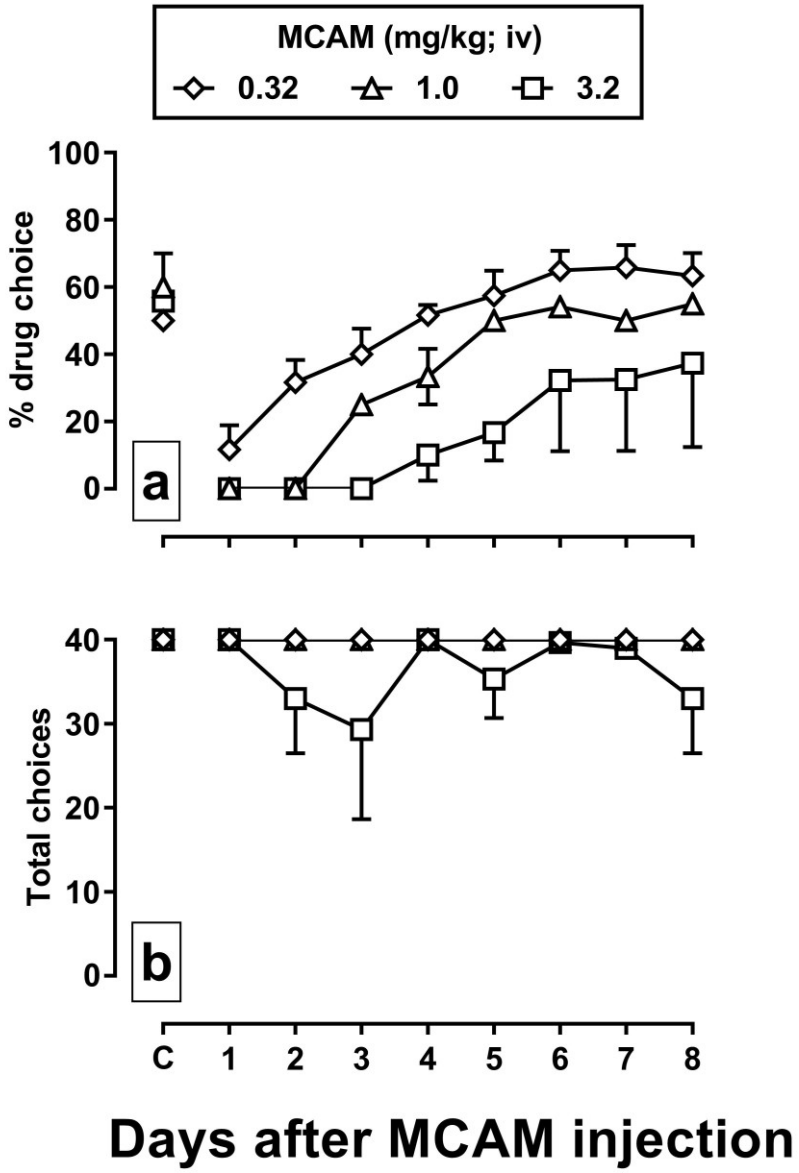

MCAM (mg/kg; iv)
0.32
1.0
3.2
% drug choice
100
80
60
40
20
0
a
Total choices
40
30
20
10
0
b
C
1
2
3
4
5
6
7
8
Days after MCAM injection

Figure 6

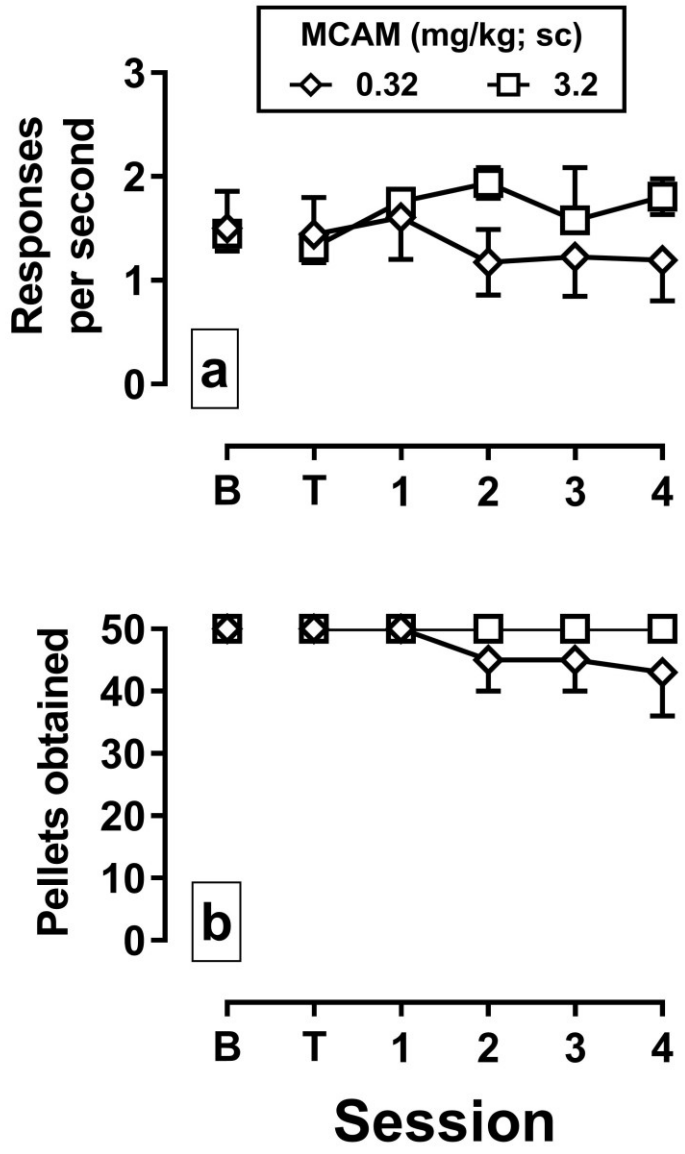
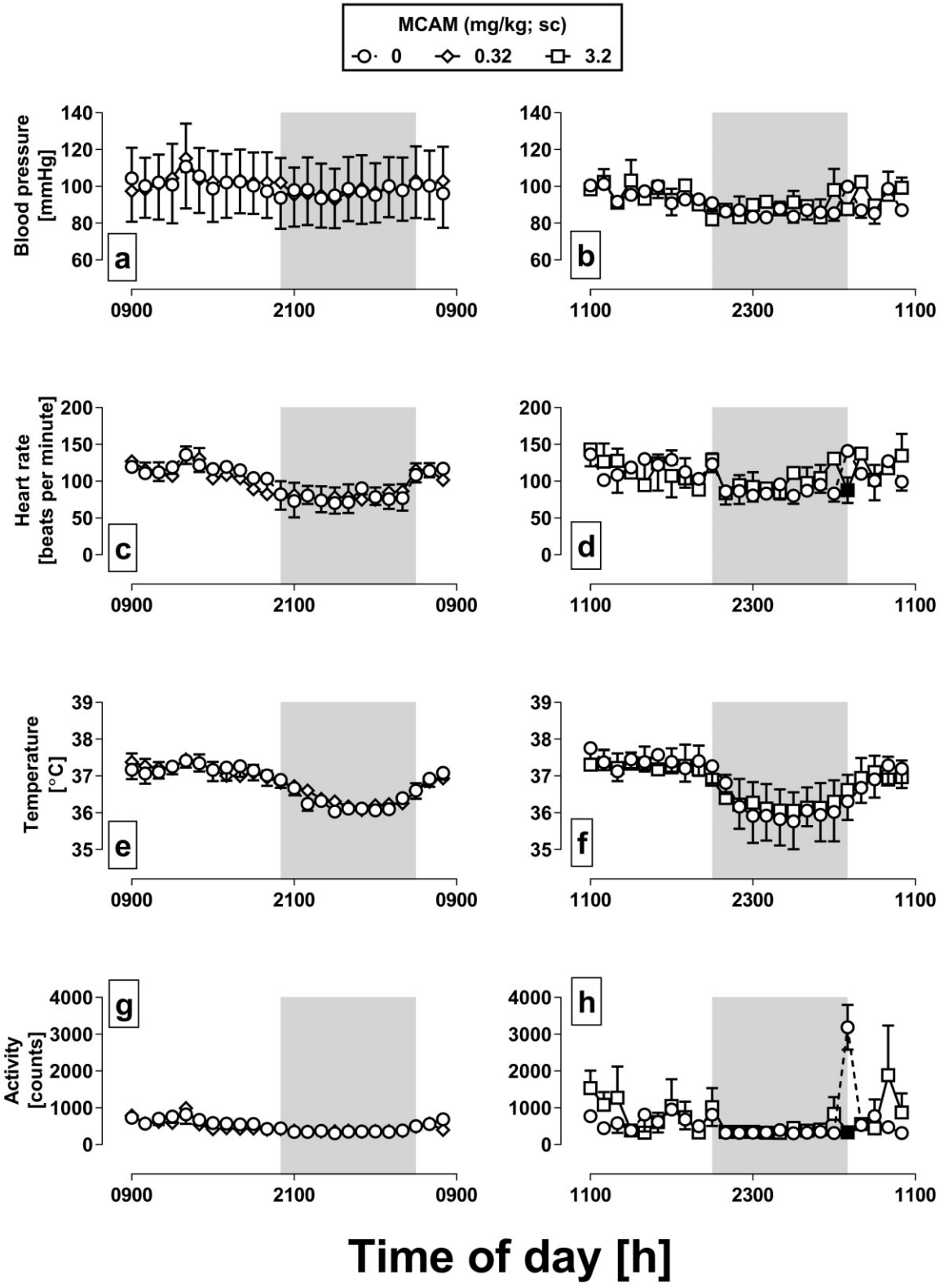

MCAM (mg/kg; sc)
0.32
3.2
Responses per second
3
2
1
0
a
B
T
1
2
3
4
Pellets obtained
50
40
30
20
10
0
b
B
T
1
2
3
4
Session

Figure 7


MCAM (mg/kg; sc)
0
0.32
3.2
Blood pressure [mmHg]
Heart rate [beats per minute]
Temperature [°C]
Activity [counts]
a
b
c
d
e
f
g
h
0900
2100
0900
1100
2300
1100
Time of day [h]