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Interactive Effects of μ-Opioid and Adrenergic-α_2 Receptor Agonists in Rats: Pharmacological Investigation of the Primary Kratom Alkaloid Mitragynine and Its Metabolite 7-Hydroxymitragynine

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Non-standard abbreviations: δ-opioid receptor (DOR), κ-opioid receptor (KOR), μ-opioid receptor (MOR), (+)-4-[(α*R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC80), (+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide (U-69,593), 2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-imidazole (RX821002), 7-hydroxymitagynine (7-OH-MG), Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), analysis of variance (ANOVA), adrenergic-α_2 ($A\alpha_2R$), Chinese hamster ovary (CHO), [D-Ala^2, D-Leu^5]-Enkephalin (DADLE), [D-Ala^2, *N*-MePhe^4, Gly-ol]-enkephalin (DAMGO), ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), fixed ratio (FR), human embryonic kidney (HEK), inhibition constant (Ki), Institutional Animal Care and Use Committee (IACUC), intraperitoneally (i.p.), intrathecally (i.t.), light-emitting diode (LED), mitragynine (MG), National Institutes of Health (NIH), orally by gavage (p.o.), percent maximum possible antinociceptive effect (% MPE), standard error of mean (SEM), subcutaneously (s.c.), Tris, $MgCl_2$, and ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (TME)

ABSTRACT

The primary kratom alkaloid mitragynine is proposed to act through multiple mechanisms, including actions at μ-opioid (MORs), adrenergic-α_2 receptors (Aα_2Rs), as well as conversion *in vivo* to a MOR agonist metabolite (i.e., 7-hydroxymitragynine). Aα_2R and MOR agonists can produce antinociceptive synergism. Here, contributions of both receptors to produce mitragynine-related effects were assessed by measuring receptor binding in cell membranes, and in rats, pharmacological behavioral effect antagonism studies. Mitragynine displayed binding affinity at both receptors, whereas 7-hydroxymitragynine only displayed MOR binding affinity. Compounds were tested for their capacity to decrease food-maintained responding and rectal temperature and to produce antinociception in the hotplate test. Prototypical MOR agonists and 7-hydroxymitragynine, but not mitragynine, produced antinociception. MOR agonist and 7-hydroxymitragynine rate-deceasing and antinociceptive effects were antagonized by the opioid antagonist naltrexone, but not by the Aα_2R antagonist yohimbine. Hypothermia only resulted from reference Aα_2R agonists. The rate-deceasing and hypothermic effects of reference Aα_2R agonists were antagonized by yohimbine but not naltrexone. Neither naltrexone nor yohimbine antagonized the rate-decreasing effects of mitragynine. Mitragynine and 7-hydroxymitragynine increased the potency of the antinociceptive effects of Aα_2R but not MOR reference agonists. Only mitragynine produced hypothermic effects. Isobolographic analyses for the rate-decreasing effects of the reference Aα_2R and MOR agonists was also conducted. These results suggest mitragynine and 7-hydroxymitragynine may produce antinociceptive synergism with Aα_2R and MOR agonists. When combined with Aα_2R agonists, mitragynine could also produce hypothermic synergism.

Significance Statement

Mitragynine is proposed to target the μ-opioid (MOR) and adrenergic-α_2 receptor (Aα_2R) and to produce behavioral effects through conversion to its MOR agonist metabolite 7-hydroxymitragynine. Isobolographic analyses indicated supra-additivity in some dose ratio combinations. This study suggests mitragynine and 7-hydroxymitragynine may produce antinociceptive synergism with Aα_2R and MOR agonists. When combined with Aα_2R agonists, mitragynine could also produce hypothermic synergism.

INTRODUCTION

Prescription μ-opioid receptor (MOR) agonists are a primary medication class to treat severe pain (Haq et al., 2021; Montgomery, 2022). However, due to the current high incidents of opioid overdose in the United States (Mattson et al., 2021), there is the need for novel analgesics that are equally effective as MOR agonists, but are safer. One of the adverse effects of MOR agonists is the development of dependence and withdrawal. The current medications to treat opioid dependence and withdrawal are either MOR or adrenergic-α_2 receptor (Aα_2R) agonists.

Mitragyna speciosa (kratom), a plant native to Southeast Asia, is used as a self-remedy to alleviate opioid withdrawal symptoms in countries such as Malaysia and Thailand (Singh et al., 2014). The use of kratom has increased significantly in the West where kratom products are used for pain reduction, opioid dependence, as well as recreationally (Lydecker et al., 2016; Sharma et al., 2019). Mitragynine (MG), the primary alkaloid in kratom, has received much attention due to its MOR activity (Matsumoto et al., 1996; Shamima et al., 2012; Harun et al., 2015; Varadi et al., 2016; Kruegel et al., 2019; Obeng et al., 2020; Obeng et al., 2021b; Chakraborty et al., 2021). However, MG appears to have a complex pharmacology that may include Aα_2R activity. For example, the antinociceptive effects of MG were reversed by both opioid (naloxone) and Aα_2R (yohimbine and idazoxan) antagonists (Matsumoto et al., 1996; Kruegel et al., 2019; Foss et al., 2020).

Decreased overreliance on prescription MOR agonists for pain management could be achieved by combining MOR agonists with non-opioid analgesics, thereby reducing the analgesic dose of the prescribed MOR agonist (i.e., opioid-sparing effect) (Wilkerson et al., 2016; Wilkerson et al., 2017; Wilkerson et al., 2019; Obeng et al., 2021a). Although the antinociceptive effectiveness of Aα_2R agonists is generally lower than that of MOR agonists, Aα_2R agonists have well-established opioid-sparing effects and have been safely used (Crassous et al., 2007; Giovannoni et al., 2009; Tonner, 2017; Valverde and Skelding, 2019). It has been hypothesized that the basis of Aα_2R agonist opioid-sparing effects is due to antinociceptive synergism (supra-additivity) between agonists at these receptors. For example, an inactive dose of the Aα_2R agonist clonidine (0.016 mg/kg) increased the antinociceptive potency of morphine four- to five-fold without producing

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tolerance in the mouse tail flick assay (Spaulding et al., 1979). The opioid-sparing effects of $A\alpha_2R$ agonists have been demonstrated regardless of rodent species (i.e. mouse and rat), antinociceptive assays (e.g. hotplate, tail pressure, and formalin), and combinations of agonists at these receptors (Drasner and Fields, 1988; Ossipov et al., 1990b; Plummer et al., 1992; Meert and Kock, 1994; Stone et al., 1997; Hao et al., 2000; Tajerian et al., 2012; Stone et al., 2014). Importantly, antinociceptive synergism was not accompanied with non-specific motor (rotarod and open field tests) or cardiovascular (pulse oximetry) disruptions (Tajerian et al., 2012; Stone et al., 2014). Additionally, the adverse effects of the $A\alpha_2R$ agonists are far less severe than those of the MOR agonists (Walker et al., 2002). In marked contrast to the MOR agonists, $A\alpha_2R$ agonists have low, if any, potential for development of abuse and dependence (Arnsten and Li, 2005; Clemow and Walker, 2014; Gowing et al., 2016) which suggests that $A\alpha_2R$ agonists may be ideal for reducing opioid use and overdose. Given the capacity of the $A\alpha_2R$ agonists to reduce opioid use as well as the agonistic activity of MG at MOR and $A\alpha_2R$ mentioned above (Matsumoto et al., 1996; Kruegel et al., 2019; Foss et al., 2020; Chakraborty et al., 2021), we hypothesized that MG mitigates opioid withdrawal through dual agonism at these receptors.

Herein, we first assessed preclinical interaction profiles of reference agonists at MOR (methadone and morphine) and $A\alpha_2R$ (lofexidine and clonidine) in rats by measuring effects of drugs on schedule-controlled responding for food, response latency in the hotplate test, and rectal temperature (Boxwalla et al., 2010). Interactions between agonists at the κ-opioid receptor (KOR, U69,593) and $A\alpha_2R$ were also investigated. The mechanism underlying the activity of these compounds was further investigated using antagonists at the MOR (naltrexone) and $A\alpha_2R$ (yohimbine). Isobolographic analyses were conducted to investigate synergism between MOR and $A\alpha_2R$ agonists. In addition, we compared the contribution of MOR and $A\alpha_2R$ to the activity of MG and 7-hydroxymitragynine (7-OH-MG), a MOR active metabolite of MG (Kruegel et al., 2019). A receptor binding assay was employed to assess affinity of test compounds at these receptors.

METHODS AND MATERIALS

Compounds. The following are sources of compounds: [^{3}H][D-Ala^2, D-Leu^5]-Enkephalin ([^{3}H]DADLE) (PerkinElmer, Boston, MA), [^{3}H][D-Ala^2, *N*-MePhe^4, Gly-ol]-enkephalin ([^{3}H]DAMGO) (PerkinElmer),

[^{3}H]RX821002 (PerkinElmer), [^{3}H]U69,593 (PerkinElmer), clonidine hydrochloride (XGen Pharmaceuticals DJB, Inc., Horseheads, NY), lofexidine hydrochloride (Sigma-Aldrich Co., St. Louis, MO), (-)-methadone hydrochloride (National Institute on Drug Abuse, Drug Supply Program, Rockville, MD), (-)-MG hydrochloride [extracted as described in Hiranita *et al* (Hiranita et al., 2019)], (-)-7-OH-MG [semi-synthesized from MG as in Obeng et al (Obeng et al., 2021b)], (-)-morphine sulfate pentahydrate (National Institute on Drug Abuse), (-)-naltrexone hydrochloride (Sigma-Aldrich Co.), U69,593 (Sigma-Aldrich Co.), and yohimbine hydrochloride (Sigma-Aldrich Co.). Dose/concentration is expressed as the weight of the salt form listed above, or as a base if no salt form is noted. For *in vitro* studies, compounds were dissolved in dimethyl sulfoxide (Sigma-Aldrich Co.) to form stock concentrations of 10 mM. For behavioral studies, a vehicle consisting of sterile water containing 5% Tween 80 (polyoxyethylenesorbitanmonooleate, Sigma-Aldrich Co.) and 5% propylene glycol (Sigma-Aldrich Co.) was used. Compounds and vehicle were administered intraperitoneally (i.p.) in a volume of 1.0—10 mL/kg per body weight. MG and vehicle were also administered subcutaneously (s.c.) and orally (p.o.) via gavage in volumes of 1.0—10 mL/kg.

In Vitro Receptor Binding Assay. [^{3}H]RX821002 (PerkinElmer) was used to label both the human adrenergic-α_{2A} and -α_{2C} receptors ($A\alpha_{2A}R$ and $A\alpha_{2C}R$) (O'Rourke et al., 1994). These two $A\alpha_2R$ subtypes were chosen because they are involved in antinociception (Brede et al., 2004). L-α-2A (ATCC® CRL11180 ™) and L-α-2C (ATCC®CRL-11181™) L-cells (American Type Culture Collection, Manassas, VA) were used for the $A\alpha_{2A}R$ and $A\alpha_{2C}R$, respectively. [^{3}H]DADLE, [^{3}H]U69,593, and [^{3}H]DAMGO were used to label the human δ-opioid receptor (DOR), KOR, MOR, respectively, as described previously (Obeng et al., 2021b). The binding assay at the opioid receptor subtypes was conducted using monoclonal opioid receptors expressed in Chinese hamster ovary (CHO) cell lines for the DOR (generous gift from Dr. Stephen J. Cutler, University of South Carolina) and MOR (PerkinElmer). The KORs (generous gift from Dr. Stephen J. Cutler, University of South Carolina) were expressed in human embryonic kidney (HEK) cells. The K_d and B_{max} values for the radioligands at each receptor subtype were first determined using a saturation assay (Table S1). The Bradford protein assay was utilized to determine and adjust the concentration of protein required for the assay (Tal et al., 1985). Ten µg of each membrane protein was separately incubated with one of the radioligands in the presence of different

concentrations of test compounds in TME [(50 mM Tris (Sigma-Aldrich), 3 mM $MgCl_2$ (Sigma-Aldrich), and 0.2 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N′*,*N′*-tetraacetic acid (EGTA, Sigma-Aldrich), pH 7.7)] buffer for 60 minutes at room temperature. The bound radioligand was separated by filtration using the Connectorate filtermat harvester for 96-well microplates (Inotech, Dietikon, Switzerland) and counted for radioactivity using a MicroBeta2 microplate counter (PerkinElmer). Specific binding at each $A\alpha_2R$ subtype was determined as the difference in binding obtained in the absence and presence of 10 μM lofexidine (Table S1). Specific binding at the DOR, KOR, and MOR was determined as the difference in binding obtained in the absence and presence of 10 μM SNC-80, U69,593, and naltrexone, respectively.

Subjects. Adult female and male Sprague Dawley rats at ten weeks old upon arrival (Taconics, Germantown, NY, N=4 per sex) were housed individually and acclimated for at least three days to a temperature- (21.9°C ± 1.9°C) and humidity-controlled (53% ± 14%) vivarium with a 12-hour light/dark cycle (lights on at 07:00 hours E.S.T. in the daylight saving time period) during which food (2918 Teklad global 18% protein rodent diets, Envigo, Frenchtown, NJ) and reverse osmosis water were available at all times. After the acclimation period, individual body weights were maintained at no less than 85% of free feeding body weight as well as no less than 2.5 of Body Conditioning Score (Ullman-Culleré and Foltz, 1999), by adjusting daily food rations. The free feeding body weight was redetermined as requested by the veterinary staff at University of Florida. Access to chow (Dustless Precision Pellets Grain-Based Rodent Diet, Bio-Serv, Frenchtown, NJ) was provided in the rats' home cages approximately 30 minutes following daily experimental sessions. In addition to chow consumption, rats consumed a maximum of fifty 45-mg sucrose pellets (Dustless Precision Pellets® 45 mg, Sucrose, Bio-Serv) available during experimental sessions for schedule-controlled responding as described below. The animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida and was in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Apparatus. The apparatus and procedures for the operant-conditioning and hotplate experiments below were as previously described (Hiranita et al., 2019; Wilkerson et al., 2019; Obeng et al., 2021b).

1) Operant Conditioning Apparatus. Eight operant-conditioning chambers (Model ENV-008; Med Associates Inc., Fairfax, VT) were used, each enclosed within a sound-attenuating cubicle equipped with a fan for ventilation and white noise to mask extraneous sounds. On the front wall of each chamber were two retractable, 5-cm-long response levers, 5 cm from the midline and 9 cm above the grid floor. A response was defined as a downward displacement of the right lever with a force approximating 0.20 N whereas the left lever was not used. Two amber light-emitting diodes (LEDs) were aligned horizontally above two levers (one LED/lever); however, only the right LED and lever were activated for the correct study. A receptacle for the delivery of 45-mg sucrose pellets (Dustless Precision Pellets® 45 mg, Sucrose, Bio-Serv) via a pellet dispenser (Model ENV-203-20; Med Associates Inc.) was mounted on the midline of the front wall between the levers and 2 cm above the floor. Each operant conditioning chamber was connected to a Dell desktop computer (Intel® Core™ i7-7700 3.60 GHz processor, 16.0 GB of RAM, Microsoft® Windows 10) through an interface (MED-SYST-8, Med Associates Inc.). Med-PC software version V (Med Associates Inc.) controlled experimental events and recorded responses. The chamber assignments remained the same for each subject throughout the study.

2) Hotplate. A square plate (Hot Plate Analgesia Meter, 1440 Analgesia Hot Plate with RS-232 Port and Software, Columbus Instruments, Columbus, OH) was surrounded by a clear acrylic cubicle with a lid. The stability of temperature on the plate surface was verified at 52°C ± 0.1°C 30 minutes prior to each use.

3) Rectal Thermometer. An uninsulated microprobe (50313 Rat Rectal Probe, Stoelting, Wood Dale, IL) and a digital thermometer (50315 Body Temperature Thermometer, Stoelting) were used to measure rectal temperature. Veterinary ophthalmic ointment (Puralube®, Dechra Veterinary Products, Overland Park, KS) was applied to the tip of the microprobe prior to each use.

In vivo procedures. The temperature, humidity, and light/dark cycle in the experimental room were equivalent to those in the vivarium. After the acclimation period to the vivarium, schedule-controlled responding experiments were conducted in the light cycle (08:00 to 11:00 hours E.S.T. in the daylight-saving time period) at the same time each day seven days per week. On drug test days, temperature and hotplate experiments were also conducted in that order (Figure 1). Prior to the start of each daily experiment, body weight was measured.

The sample size of each experimental group per treatment was eight using a within-subject design (N=4 per sex). The doses of each test compound per injection were incremented sequentially at approximately 20-minute intervals (Figure 1).

1) Within-Session, Six-Cycle Schedule-Controlled Responding. A) Lever-response shaping. Each experimental session commenced by placing an experimental subject in an individually assigned chamber daily up to 120 minutes. Each session started with the presentation of the right retractable lever and the illumination of the LED above the right lever. Each downward deflection of the right lever turned off the LEDs and activated the pellet dispenser for 0.1 seconds [fixed-ratio (FR) 1 schedule] followed by a 0.1-second time-out period during which LEDs were turned off and responding had no scheduled consequences; the retractable lever remained presented during this time-out time. After 50 reinforcers per session were presented within 20 minutes for two consecutive sessions under the terminal FR10 schedule of reinforcement, and daily sessions were divided into multiple, discrete cycles.

B) Training. Each session consisted of six, 20-minute cycles with each cycle consisting of a 15-minute pretest phase and a five-minute test phase in the operant-conditioning chambers (Figure 1). Immediately prior to each cycle, vehicle was injected i.p. and each animal was placed in the assigned chamber. Upon commencement of each session and at the beginning of each pretest phase, the right response lever was extended into the chamber but the stimulus light remained off. Responses on the lever had no scheduled consequences. Upon commencement of each test phase of the cycle, the stimulus light was illuminated. Thereafter, all the experimental variables for the stimulus changes and response timeout conditions under the FR10 schedule of reinforcement were identical to those for response shaping except that the maximal number of food reinforcers delivered was fixed at ten per cycle. When 10 food reinforcers were delivered during each test phase, the stimulus light was turned off and lever responding had no scheduled consequences. Upon completion of the last test phase, the lever was retracted and the stimulus light was turned off. Then, each animal was placed back to their home cages. Training continued until overall response rates (responses per second) across six

cycles for two consecutive sessions were stably maintained with less than 25% variation, as determined per individual subject.

C) Testing. All the experimental variables were identical to those for the training period. However, a dose of a test compound was also injected per cycle other than vehicle. The first injection received was either vehicle or the pretreatment compound (i.e. antagonists naltrexone or yohimbine). The subsequent five injections were either vehicle or test compound. Each test compound was dosed cumulatively such that each dose per cycle was a subtraction from a summation of all the previous doses administered to achieve the target dose. The doses of the compounds administered (mg/kg) increased by either quarter or half log unit increments. Each test session was separated by a minimum of 72 hours and was studied with a non-systematic order of compounds and doses. During the inter-test maintenance sessions, all the experimental variables were identical to those for the training period, without any determination of the hotplate latency and rectal temperature as described immediately below. Vehicle was injected at the beginning of each pretest phase.

Among food-maintained behavior, hotplate response latency, and rectal temperature, only analyses of food-maintained behavior allowed to determine ED_{50} values of all the reference agonists at MOR, KOR, and $A\alpha_2R$ (see *Data analysis*). For the combinations of reference agonists, the cumulative doses in quarter log units in the mixtures per animal were determined based on the ED_{50} values of the rate-decreasing effects of reference agonist alone (Table 1), (Wilkerson et al., 2019). To determine the pharmacological influence of each drug on the observed effects, three ED_{50} ratios of drug mixtures were used. The order of testing was determined randomly. All dose-effect functions for drug mixtures were singly determined.

2) Hotplate and Rectal Temperature. On drug test days, the microprobe tip was inserted approximately 2.0-cm into each subject's rectum and individual baseline temperature was measured within 10 seconds. Immediately after the baseline measurement of rectal temperature, each subject was manually placed on the heated plate and baseline hotplate response latency was determined manually using a stopwatch (Martin Stopwatch, Martin Sports, Carlstadt, NJ) by trained and experimentally blinded raters. Hotplate response latency was measured until the subject jumped, licked or shook the back paws, or up to 60 seconds to avoid tissue damage, whichever occurred first.

Immediately following the determination of the baseline values, each subject underwent an injection of a dose of a test compound or vehicle and was placed in their respective operant conditioning chamber. Immediately after each cycle of the schedule-controlled responding experiment (cycles 1 to 6), rectal temperature and hotplate response latency were measured followed by an injection of a dose of the test compound or vehicle in this order.

Data analysis. The dependent variables in each figure are shown as mean values ± standard error of the mean (SEM). Mean and SEM values per group of eight subjects were calculated as a function of compound doses, cycles, or dose ratios of combined compounds. Statistical analyses were conducted using GraphPad Prism version 9 for Windows (GraphPad Software, Inc., San Diego, CA), SigmaPlot version 14.0 (Systat Software Inc., San Jose, CA), or R-4.1/RStudio Desktop (R Core Team, 2017). Comparisons were considered significant when a *P* value was less than 0.05. A one-, two-, or three-way (repeated-measures) analysis of variance (ANOVA) followed by *post hoc* Bonferroni *t* tests was used as appropriate to analyze the effects of the compound dose, cycle, sex, dose ratio, or tolerance (assessment order: first or last dose-effect assessment for morphine, U69,593, and lofexidine; Figure S2 and Table S5-S7). For the three-way repeated measures ANOVA, GraphPad Prism software was used for all 2 by 2 by X design and the RStudio Desktop software was used for all others.

For rectal temperature and hotplate latency, each mean baseline value was determined per animal from all the baseline values determined on the drug sessions used in the following analyses. Hotplate latency values were converted to percent maximum possible antinociceptive effect (% MPE) with the following equation: (100 × [(experimental test latency value – the averaged baseline latency value) / (60 seconds – the averaged baseline latency value)]). Changes in rectal temperature were calculated individually as the test value subtracted from the averaged baseline value. Rates of responding maintained by presentations of food pellets (responses/second) were expressed as a percentage of control, defined as the mean baseline rates across six daily cycles during all sessions one day prior to each test session. There was no increased or decreased trend for either hotplate latency, rectal temperature, or response rate baseline values (P values > 0.05). The dose-

effect functions of morphine, U69,593, and lofexidine were determined twice, once at the start and once at the end of the within-subjects drug assessments. Only when the mean effect of a compound to reduce schedule-controlled responding or to increase MPE was greater than 50% of maximum effects were the ED_{50} and slope values calculated using multiple linear regression (Snedecor and Cochran, 1967) and GraphPad Prism version 9 for Windows (GraphPad Software), where slopes were allowed to vary (Tallarida, 2000). Because only α_2R agonists produced 2°C or greater hypothermia, $ED_{-2°C}$ values were also individually calculated to compare the hypothermic potency. Only points on the linear part of the ascending (%MPE) and descending (response rate and rectal temperature) limbs of the dose–effect functions were used. If the 95% confidence intervals (CIs) of the ED_{50}, $ED_{-2°C}$, and slope values did not overlap, or the potency or slope ratio of the compound alone or in combination with another compound did not include 1, potencies or slopes of the compounds were deemed statistically different. Among food-maintained behavior, hotplate response latency, and rectal temperature, only analyses of food-maintained behavior allowed to determine ED_{50} values of all the reference agonists at MOR, KOR, and Aα_2R. For the mixture studies, the cumulative doses in quarter log units in the mixtures per animal were determined based on the ED_{50} values of the rate-decreasing effects of reference agonist alone (Wilkerson et al., 2019). That is, a within-subjects design was used, and each subject received dose combinations that were equivalent to the dose ratio based upon the individual ED_{50} of a drug to decrease response rates in that subject. The theoretical additive ED50 value of the combined drugs was calculated from the individual dose-effect functions to determine synergistic, additive, or subadditive interactions as previously described (Wilkerson et al., 2016; Wilkerson et al., 2017; Wilkerson et al., 2019). The combination was assumed to equal the sum of the effects of each drug. The experimentally derived ED50 values (Zmix) from the dose-effect functions of the ratios were compared to the predicted additive ED50 values (Zadd) via a Fisher's exact test (Wilkerson et al., 2016; Wilkerson et al., 2017; Wilkerson et al., 2019). If the empirically derived value and the theoretical value did not significantly differ, the interaction was considered additive (Tallarida, 2001; 2006). For the *in vitro* studies, the assays were conducted in triplicate and repeated at least three times and the IC_{50} values were determined using a nonlinear, least-squares regression analysis (Prism 9;

GraphPad Software, Inc.) and then converted to K_i values using the Cheng–Prusoff equation (Cheng and Prusoff, 1973). The 95%CI (asymptotic) was calculated using Prism 9.

RESULTS

Only the primary findings are shown here. Full details are described in the supplemental materials.

Receptor Binding. The Ki (nM) values of reference Aα_2R ligands clonidine, lofexidine, and yohimbine were 5.97, 1.21, and 8.24 at the Aα_{2A}R, and 60.8, 7.62, and 7.77 at the Aα_{2C}R, respectively (Table 1). The Ki values of reference Aα_2R ligands at opioid receptor subtypes and of reference opioid receptor ligands (methadone, morphine, naltrexone, and U69,593) at Aα_2R subtypes were not determined due to lack of inhibition up to 10 μM (Table 1). The Ki values of MG were 4,420 and 4,040 nM at the Aα_{2A}R and Aα_{2C}R, respectively, whereas those of 7-OH-MG at these receptors were not determined due to lack of inhibition up to 10 μM. Both MG and 7-OH-MG had higher affinities at the MOR than at the DOR and KOR; however, 7-OH-MG had a 9-fold higher affinity at the MOR than MG (Figure 2 and Table 1). A summary of scintillation counting conditions employed for assessing affinity at various binding sites in competition for the radioligands labeling human Aα_2R and opioid receptor subtypes can be found in Supp. Table 1.

Reference MOR Agonists Alone. Repeated vehicle injections did not alter response rates, rectal temperature, or nociceptive responding (Supp. Fig. 1, Supp. Table 2, Supp, Table 3). Morphine dose-dependently and significantly decreased response rates and rectal temperature, as well as produced antinociception (Figure 3, upper panels, upward triangles; Supp. Table 4). The ED_{50} values of morphine to decrease response rates and to produce antinociception are shown in Table 2. The potency of morphine to produce the rate-decreasing effects was 4-fold more potent than that for antinociception (Table 2).

Methadone significantly decreased response rates and rectal temperature, and produced antinociception (Figure 3, upper panels, downward triangles; Supp. Table 4, Supp. Table 5). Relative to morphine, methadone was 7- and 5-fold more potent to produce rate-decreasing and antinociceptive effects, respectively (Table 2).

Reference KOR Agonist Alone. U69,593 significantly decreased response rates and rectal temperature, and produced antinociception (Figure 3, upper panels, circles; Supp. Table 6). Relative to morphine, U69,593 was 2- and 4-fold more potent to produce the rate-decreasing and antinociceptive effects, respectively (Table 2). U69,593 was equipotent to decrease response rates and produce antinociception, as measured by increased %MPE (Table 2). There was no significant change in potency across the rates of responding, antinociception, or rectal temperature (Supp. Figure 2; Table 2 and Supp. Table 6).

Reference Aα_2R Agonists Alone. Lofexidine significantly decreased response rates and rectal temperature, and significantly increased %MPE; the antinociceptive effects of lofexidine reached statistical significance but the maximum effects of lofexidine were a mean of 17.3% and significantly less than those of reference MOR agonists ($F_{1,6}$=361, P<0.001, two-way repeated measures ANOVA; Figure 3, upper panels, diamonds; Supp. Table 7). In contrast, as compared to the reference MOR agonists, the hypothermic effects of lofexidine were significantly greater, e.g., 4.1°C decrease in rectal temperature at 0.56 mg/kg (Figure 3). Lofexidine was 38-fold more potent than morphine to produce the rate-decreasing effects (Table 2). The potency of lofexidine to reduce response rates was 3-fold greater than its potency to decrease rectal temperature (Table 2).

Clonidine significantly decreased response rates and rectal temperature; however, statistically significant antinociception was not obtained (Figure 3, upper panels, squares; Supp. Table 7). Clonidine was 4- and 3-fold more potent than lofexidine to produce the rate-decreasing and hypothermic effects, respectively (Table 2). The potency of clonidine to produce the rate-decreasing effects was 4-fold more potent than that for the hypothermic effects (Table 2).

MG and 7-OH-MG Alone. When administered i.p., MG significantly decreased response rates; however, neither statistically significant antinociception nor altered rectal temperature was obtained (Figure 3, lower panels, circles; Supp. Table 8). MG (i.p.) was 4-fold more potent than i.p. morphine to produce the rate-decreasing effects (Table 2). MG had been expected to produce antinociceptive and hypothermic effects because other effects produced by MG are antagonized by MOR and A_2R antagonists (Foss et al., 2020; Obeng

et al., 2021b). Thus, the route of administration of MG was varied and the effects of 7-OH-MG, an active metabolite of MG at the MOR, were assessed.

Both p.o. and s.c. MG significantly decreased rates of responding, and no significant antinociception was observed; there were relatively small yet significant increases in rectal temperature (Figure 3, lower panels, downward and upward triangles, respectively; Supp. Table 8). MG administered p.o. and s.c. was 3- and 6-fold less potent, respectively, than i.p. MG to produce the rate-decreasing effects (Table 2).

In contrast to MG, i.p. 7-OH-MG significantly decreased response rates and produced hot plate antinociception; however, no significant effects on rectal temperature were obtained (Figure 3, lower panels, squares; Supp. Table 8). The potency of 7-OH-MG to reduce response rates was approximately 4-fold more potent than its potency to produce antinociception (Table 2).

Reference MOR Agonists in Combination with Naltrexone or Yohimbine. By themselves, naltrexone (0.032, 1 mg/kg, i.p.) and yohimbine (1, 3.2 mg/kg, i.p.), did not alter food-maintained behavior, antinociception, or rectal temperature (Supp. Fig. 3, and Supp. Table 9). Naltrexone dose-dependently and significantly shifted to the right the dose-effect functions of the rate-decreasing and antinociceptive effects of morphine (Figure 4; Table 2, and Supp. Table 4). The lower dose of naltrexone (0.032 mg/kg) produced significant antagonism of the rate-decreasing and antinociceptive effects of morphine (Table 2). Yohimbine (3.2 mg/kg) did not significantly change the effects of morphine on rates of responding, antinociception, or changes in rectal temperature (Figure 4; Table 2, and Supp. Table 4).

Naltrexone (0.032 mg/kg) produced a 5-fold rightward shift of the methadone rate-decreasing dose-effect function (Figure 4; Tables 2, and Supp, Table 4). Yohimbine (3.2 mg/kg) did not significantly modify the effects of methadone on rates of responding, antinociception, or changes in rectal temperature (Figure 4; Table 2, and Supp. Table 4).

U69,593 in Combination with Naltrexone or Yohimbine. Naltrexone (0.032 mg/kg) produced a small but statistically significant leftward shift of the U69,593 rate-decreasing dose-effect function, but did not modify U69,593 antinociceptive or hypothermic effects (Figure 4; Table 2, and Supp. Table 6). Naltrexone (1.0

mg/kg) significantly antagonized the rate-decreasing, antinociceptive, and hypothermic effects of U69,593 (Figure 4; Table 2, and Supp. Table 6). Naltrexone produced a 5- and 3-fold, respectively, rightward shift of the U69,593 rate-decreasing and antinociceptive dose-effect function (Table 2). Yohimbine (3.2 mg/kg) did not modify U69,593-related rates of responding, antinociception, or rectal temperature (Figure 4; Table 2, and Supp. Table 6).

Reference Aα_2R Agonists in Combination with Naltrexone or Yohimbine. Naltrexone did not modify the effects of lofexidine on rates of responding, hot plate antinociception, or rectal temperature (Figure 4; Table 2 and Supp. Table 7). Yohimbine dose-dependently and significantly shifted to the right the dose-effect functions of the rate-decreasing and hypothermic effects of lofexidine (Figure 4; Table 2 and Supp. Table 7). The lower dose of yohimbine (1.0 mg/kg) produced a 4-fold shift to the right of the lofexidine dose-effect functions to decrease response rates and rectal temperature (Supp. Table 7).

Naltrexone did not modify the effects of clonidine on rates of responding, antinociception, or rectal temperature (Figure 4; Table 2, and Supp. Table 7). Yohimbine (1.0 mg/kg) produced an 8- and 4-fold, respectively, rightward shift of the clonidine rate-decreasing and hypothermic dose-effect function (Figure 4; Table 2 and Supp.Table 7).

MG (i.p.) and 7-OH-MG in Combination with Naltrexone or Yohimbine. Because the i.p. route was most potent among the three routes of administration tested in decreasing the response rates, the i.p. route was used to assess the pharmacological impact of naltrexone (1.0 mg/kg) or yohimbine (3.2 mg/kg) on MG-related behaviors and physiology. Neither naltrexone nor yohimbine significantly modified the dose-effect function of MG to decrease responding (Figure 5; Table 2 and Supp. Table 8). Naltrexone (0.032 mg/kg) significantly shifted the dose-effect functions of 7-OH-MG 3-fold rightward for both rate-decreasing and antinociceptive effects (Figure 5; Table 2 and Supp. Table 8). In contrast, yohimbine (3.2 mg/kg) did not significantly modify the rate-decreasing or antinociceptive 7-OH-MG dose-effect functions (Figure 5, Table 2, and Supp. Table 8).

Reference Agonists in Combination with MG or 7-OH-MG. By themselves, MG (17.8 mg/kg, i.p.) and 7-OH-MG (0.32 mg/kg, i.p.), did not alter food-maintained behavior, antinociception, or rectal temperature (Supp. Fig. 3, and Supp. Table 9). Pretreatment effects of behaviorally inactive doses of MG (17.8 mg/kg) or 7-OH-

MG (0.32 mg/kg) were assessed on the effects of reference agonists tested above in order to understand the interaction of MG or its metabolite with the reference agonists (Figure 6). Neither MG nor 7-OH-MG significantly modified the rate-decreasing and antinociceptive dose-effect functions of morphine and methadone (Figure 6, Table 2, and Supp. Table 4).

MG pretreatment did not significantly modify the rate-decreasing, antinociceptive, and hypothermic dose-effect functions of U69,593 (Figure 6; Table 2 and Supp. Table 6). 7-OH-MG did not significantly alter the dose-effect functions of rates of responding or rectal temperature for U69,593 whereas 7-OH-MG produced a significant 4-fold rightward shift in the U69,593 hotplate antinociception dose-effect function (Figure 6, Table 2, and Supp. Table 6).

MG produced a leftward shift in both lofexidine and clonidine rate-decreasing and hypothermic effect dose-effect functions (Figure 6; Table 2 and Supp. Table 7). When combined with MG, lofexidine and clonidine produced significantly greater hotplate antinociception than either lofexidine alone or clonidine alone (Figure 6; Table 2 and Supp. Table 7). The mean hotplate antinociceptive values, expressed as %MPE, of lofexidine alone and clonidine alone were less than 20% (Figure 6). As with MG, 7-OH-MG shifted to the left the dose-effect functions of the rate-decreasing effects of lofexidine and clonidine and rendered lofexidine and clonidine antinociceptive (Figure 6; Table 2 and Supp. Table 7). However, and in contrast to MG, 7-OH-MG did not significantly modify either lofexidine or clonidine hypothermic dose-effect functions (Figure 6; Table 2 and Supp. Table 7).

Combinations of the Reference Agonists. Among food-maintained behavior, hotplate response latency, and rectal temperature, only analyses of food-maintained behavior were used to determine the ED_{50} values of all the reference agonists at MOR, KOR, and Aα_2R (Table 3). Based on the calculated rate decreasing ED_{50} values of each reference compound alone, doses for the mixtures in ED_{50} ratios of 3:1, 1:1, and 1:2 parts morphine to lofexidine, were administered cumulatively in quarter log units (Table 3). Each drug combination produced dose-related decreases in response rates (Supp. Fig. 4; Supp. Table 10). Hotplate antinociception and hypothermia were also assessed. All morphine dose ratios produced similar leftward antinociceptive morphine dose-effect function shifts. As the morphine dose ratio increased (i.e., 1:2, 1:1, 3:1 morphine to lofexidine) the

hypothermia dose-effect functions shifted further to the left (Supp. Fig. 4; Supp. Table 10). As the lofexidine dose ratio *decreased* (i.e., 1:2, 1:1, 3:1 morphine to lofexidine) the antinociception dose-effect functions shifted further to the left (Supp. Fig. S4; Supp. Table 10). All lofexidine dose ratios produced similar leftward lofexidine hyperthermic dose effect function shifts.

We also examined, based upon the ED_{50} doses to decrease response rates, 2:1, 1:2, and 3:1 morphine to clonidine dose mixtures. Each drug combination produced dose-related decreases in response rates. We found similar shifts as seen with morphine and lofexidine, in the morphine and clonidine antinociceptive and hypothermia dose-effect relationships (Supp. Fig. 5; Supp. Table 10). A similar trend for inverse opioid and adrenergic receptor agonist antinociceptive and hypothermic dose-effect function shifts, based on the relative opioid to adrenergic receptor agonist dose ratio were also consistently observed with 1:2, 1:1, 3:1 methadone to lofexidine (Supp. Fig. 6; Supp. Table 10), 4:1, 2:1, 1:1 methadone to clonidine (Supp. Fig. 7; Supp. Table 10), 1:2, 1:1, 2:1 U69,593 to lofexidine (Supp. Fig. 8; Supp. Table 10) and 1:2, 2:1, 3:1 U69,593 to clonidine (Supp. Fig. 9; Supp. Table 10) ED_{50} ratios.

Interactive Effects of Reference Compounds. Sub-additivity for drug combination rate decreasing effects was not observed in any of the above discussed morphine to lofexidine, morphine to clonidine, methadone to lofexidine, methadone to clonidine, U69,593 to lofexidine, or U69,593 to clonidine drug combinations (Figure 7; Table 4). Additive effects were generally observed, with a few exceptions where supra-additivity was found. Supra-additivity was observed under the following dose ratios 1:1 and 1:2 morphine to lofexidine, 2:1 and 1:2 morphine to clonidine, 2:1 methadone to clonidine, 1:1, 1:2, 2:1 U69,593 to lofexidine, 2:1, 1:2 U69,593 to clonidine (Figure 7; Table 4).

DISCUSSION

In this study we observed several novel findings. MG had comparable binding affinities at $A\alpha_2R$ and MOR whereas 7-OH-MG, an active metabolite of MG, had relatively high affinity at MOR and negligible affinity at $A\alpha_2R$. Among three experimental assays employed in this study, we examined drug-drug schedule-controlled responding interactions via isobolar analysis. MG and 7-OH-MG potentiated the rate-decreasing effects of $A\alpha_2R$ agonists, but not MOR agonists, and increased the potency of $A\alpha_2R$ agonists to produce

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antinociception. MG but not 7-OH-MG potentiated the hypothermic effects of the $A\alpha_2R$ agonists. Neither naltrexone nor yohimbine antagonized the rate-decreasing effects of MG, whereas naltrexone, but not yohimbine, antagonized the rate-deceasing effects of 7-OH-MG. Thus, these isobolar analyses suggest that to produce the opioid-sparing effects of $A\alpha_2R$ agonists a specific dose combination is required. In addition, these results suggest that MG and 7-OH-MG may produce antinociceptive synergism with both $A\alpha_2R$ and MOR agonists. Furthermore, MG but not 7-OH-MG when combined with $A\alpha_2R$ agonists may produce hypothermic synergism.

The supra-additive interactions between MOR and $A\alpha_2R$ on schedule-controlled responding was observed at various dose ratios (i.e., 2:1, 1:1, 1:2) and these interactive effects may be specific to schedule-controlled responding. For example, in several mouse and rat antinociception studies others have found supra-additive interactions between MOR and $A\alpha_2R$ only when mixtures included low proportions of the MOR agonist relative to an $A\alpha_2R$ agonist based on their individual potencies (Spaulding et al., 1979; Drasner and Fields, 1988; Tajerian et al., 2012; Stone et al., 2014). Additionally, our findings demonstrate that schedule-controlled responding supra-additive interactions at $A\alpha_2R$ were not pharmacologically specific for MOR, as supra-additive interactions with $A\alpha_2R$ agonists were observed with the KOR agonist U69,593. These results highlight the importance of the proportions of MOR agonists in complex drug mixtures on observed behavior. An additional consideration for these studies is that here we only examine schedule-controlled responding drug-drug interactions via isobolar analysis. Although we additionally studied hotplate antinociception and hypothermia in these animals, we are unable to determine if these observed dose-response function shifts were subadditive, additive or supra-additive. Additional experiments beyond the scope of the current study would identify antinociceptive and hypothermic drug-drug additivity interactions.

Although not explicitly examined in the present study, supra-additive antinociception resulting from combinations of $A\alpha_2R$ and KOR agonists has been reported (Ossipov et al., 1990a; Roerig, 1994). Specifically, supra-additive antinociception was produced in rats using a tail withdrawal assay when three parts of clonidine and one part of U69,593 were administered intrathecally (i.t.) (Ossipov et al., 1990a). Further, supra-additive

antinociception was produced in mice using the tail withdrawal assay when one part of clonidine and one part of the KOR agonist U50-488H were administered intrathecally (i.t.) (Roerig, 1994). When compared to our additive KOR and Aα_2R schedule-controlled responding behavioral findings in rats, there are a number of differences across the present and previous studies that may contribute to the observed differences in additive vs. supra-additive drug effects (Ossipov et al., 1990a; Roerig, 1994); assays employed (i.e., antinociception vs. schedule-controlled responding), the routes of administration of compounds (i.e., i.p. versus i.t.), and drug history (i.e. a complex drug history versus naive). These differences may individually and combined yield different receptor densities and receptor pools that mediate the underlying observed behavioral results.

The affinities of MG at both MOR and Aα_2R were approximately equal whereas the affinity of 7-OH-MG was high at the MOR (77.9 nM) and negligible at the Aα_2R. In our studies, MG failed to mimic the antinociceptive effects of MOR agonists or the hypothermic effects of Aα_2R agonists. These findings are in contrast to previously reported results which demonstrated that MG produced antinociceptive effects in C57BL/6J mice (Chakraborty et al., 2021). Additionally, neither naltrexone nor yohimbine antagonized mitragynine-induced decreases in food-maintained behavior. Under the same experimental conditions, naltrexone antagonized the effects of MOR agonists, and yohimbine antagonized the effects of Aα_2R agonists. In contrast to MG, 7-OH-MG mimicked the effects of morphine and methadone. Superficially, these MG results suggest no contribution of the MOR or Aα_2R to the pharmacological effects of MG in rats. However, as the discriminative-stimulus effects of MG in rats were antagonized by naltrexone, our current results do not broadly apply to all in vivo pharmacological assessments (Obeng et al., 2021b). Additionally, in a neuropathic pain model, the anti-allodynic effects of MG in rats were antagonized by yohimbine (Foss et al., 2020). The inability of naltrexone to antagonize the rate-decreasing effects of MG has previously been reported (Hiranita et al., 2019; Obeng et al., 2021b). Naltrexone was 3.2-fold less potent in antagonizing the rate-decreasing effects of morphine than in antagonizing the discriminative-stimulus effects of morphine in rats (Obeng et al., 2021b). Thus, the sensitivity to the pharmacological activity of interest differs across experimental assays employed.

Both MG and 7-OH-MG potentiated the rate-decreasing effects of lofexidine and clonidine, but not those of morphine and methadone, and increased the maximum antinociceptive effects of the $A\alpha_2R$ agonists. However, MG, but not 7-OH-MG, potentiated the hypothermic effects of the reference $A\alpha_2R$ agonists. The MG-induced potentiation of the hypothermic and antinociceptive effects of the reference $A\alpha_2R$ agonists might suggest positive allosteric effects of MG at the $A\alpha_2R$; however, there is currently no such published report or supportive evidence. Nonetheless, there are clinical implications, in that, MG can be used to enhance the clinical effects of $A\alpha_2R$ agonists; such as pain relief as well as the ability to block the acute withdrawal symptoms in chronic opioid users. Additionally, the in vivo "apparent" positive allosteric effects of MG at the $A\alpha_2R$ might indicate a challenging hypothesis that MG could mitigate opioid withdrawal (Wilson et al., 2020; Wilson et al., 2021) primarily due to allosteric agonism at the $A\alpha_2R$ rather than dual agonism at the MOR and $A\alpha_2R$ (Chakraborty et al., 2021). It is worth noting that MG is metabolized by CYP3A4 to 7-OH-MG (Kamble et al., 2019; Basiliere and Kerrigan, 2020; Chakraborty et al., 2021). It was recently reported that metabolic conversion of 7-OH-MG does not contribute to MG pharmacological activity (Berthold et al., 2022). However, other studies showed that 7-OH MG does contribute to the analgesic and respiratory depressive effects of MG, albeit its contribution was found to be limited by metabolic saturation (Kruegel et al., 2019; Chakraborty et al., 2021; Hill et al., 2022). In the study by Berthold and colleagues it was demonstrated that in mice treated with MG doses which produced significant hotplate antinociception, 7-OH-MG brain levels remained significantly below the observed 7-OH-MG brain levels found in 7-OH-MG treated mice that were dosed sufficiently to produce acute antinociception (Berthold et al., 2022). In this study, the pharmacological activity of 7-OH-MG was quite different from that of MG, which contradicts the hypothesis that 7-OH-MG is responsible for the "apparent" antinociceptive effects of MG in mice (Kruegel et al., 2019). The inconsistency between the present and previous (Kruegel et al., 2019) studies might simply be due to a difference in species (i.e., rat vs. mouse, respectively).

To assess the therapeutic utility of these kratom alkaloids, future studies should examine the sub-additive, additive vs. supra-additive effects of MG, 7-OH-MG, and MOR as well as $A\alpha_2R$ agonists in relevant pathological pain and drug dependence models. In conclusion, supra-additive interaction between agonism at

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the MOR and Aα_2R depend on the dose combination ratio and MOR agonist used. Affinity of MG at these receptors was approximately equal whereas no considerable affinity of 7-OH-MG was found at the Aα_2R.

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Contributed new reagents or analytic tools: Leon and McCurdy.

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Wrote or contributed to the writing of the manuscript: Obeng, Leon, Zuarth Gonzalez, Da Silva, Shiomitsu, Soto, McCurdy, McMahon, Wilkerson, and Hiranita.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. Schematic presentation of experimental timelines on test and inter-test sessions. The rate-decreasing, hypothermic, and antinociceptive effects of test compounds were repeatedly assessed in eight rats (four rats per sex) by measuring schedule-controlled responding (SCR) for presentation of food pellets, rectal temperature (RT), and hotplate (HP) response latency, respectively. RT and HP response latency were measured manually in this order only on test days. RT was measured using a microprobe. HP response latency was measured by placing each rat on a heated hotplate at 52°C and using a stopwatch. The experimental session consisted of six 20-minute experimental cycles and lasted for 120 minutes. On the test days, baseline values of RT and HP response latency were measured before the experimental session. After each rat received an injection (i.p., p.o., or s.c.) (T=0 minutes), the first experimental cycle commenced by placing the rat in the operant-conditioning chamber. Each experimental cycle consisted of the 15-minute timeout period and then 5-minute period for data collection of lever-pressing responses for presentations of food pellets using an automated system. Immediately following each 20-minute cycle, RT and HP response latency were measured in this order. Then, each rat received an injection of a dose of test compound and the second cycle commenced by placing the rat in the operant-conditioning chamber. Doses of each test compound was administered cumulatively. The experimental procedures on inter-test days were basically identical to those on test sessions.

However, RT and HP response latency were not measured on inter-test days. In addition, only vehicle was administered on inter-test days. The inter-test sessions were conducted consecutively at least twice. See Methods section for more details.

Figure 2. Displacement of radioligands at opioid receptor and Aα_2R subtypes. Ordinates: percentage of specific radiotracer bound to membrane preparations. Abscissae: concentrations of each competing compound (log scale). Each data point represents the mean results of three repeated experiments; vertical bars represent S.E.M. (N = 3) from at least three independent triplicate replications per sample. Ki and 95% CI values from curve-fitting analyses of these data are shown in Table 1. Note that affinity of MG at the MOR and Aα_2R was approximately equal whereas no considerable affinity of 7-OH-MG was found at the Aα_2R.

Figure 3. The rate-decreasing, antinociceptive, and hypothermic effects of various compounds alone in rats. Abscissae: Vehicle and cumulative dose of compound in mg/kg (log scale). Ordinates: *Left panels*, percentage of mean rates of responding after repeated administration of vehicle during inter-test sessions; *middle panels*, percentage of maximum possible effect (%MPE) in the hotplate assay; *right panels*, changes in rectal temperature from mean baselines. Each point represents the mean ± SEM (N=4 per sex per data point). All compounds were administered i.p. 15 minutes before each 5-minute period for data collection for food-maintained behavior and MG was also administered p.o. and s.c. (lower panels). The data for morphine, U69,593, and lofexidine on the first assessment were plotted. *Upper left*: The rate-decreasing effects of vehicle, the reference MOR agonists (morphine and methadone), reference Aα_2R agonists (lofexidine and clonidine), and reference KOR agonist U69,593. Filled circles represent repeated vehicle (i.p.) administration. Morphine dose (i.p., upward triangles); vehicle, 5.6, 10, 17.8, 32, and 56 mg/kg. Methadone dose (i.p., downward triangles); vehicle, 0.32, 0.56, 1.0, 1.78, and 3.2 mg/kg. Lofexidine doses (i.p., diamonds); vehicle, 0.056, 0.1, 0.178, 0.32, and 0.56 mg/kg. Clonidine doses (i.p., squares); vehicle, 0.0178, 0.032, 0.056, 0.1, and 0.178 mg/kg. U69,593 doses (i.p., open circles); 0.56, 1.0, 1.78, 3.2, and 5.6 mg/kg. *Upper middle*: The antinociceptive effects of reference compounds. *Upper right*: The hypothermic effects of reference compounds. *Lower left*: The rate-decreasing effects of MG and 7-OH-MG. MG dose (i.p., circles); vehicle, and 5.6, 10, 17.8, 32, and 56 mg/kg. MG dose (p.o., circles); vehicle, 17.8, 32, 56, 100, and 178 mg/kg. MG dose

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(s.c., triangles); vehicle, 17.8, 32, 56, 100, and 178 mg/kg. 7-OH-MG dose (i.p., squares); vehicle, 0.32, 1.0, 3.2, 10, and 32 mg/kg. *Lower middle*: The antinociceptive effects of MG and 7-OH-MG. *Lower right*: The hypothermic effects of MG and 7-OH-MG. Each gray symbol indicates a significant difference from vehicle per corresponding cycle. Note that all test compounds decreased food-maintained behavior. Robust antinociception was produced by the reference MOR agonists but not by the reference Aα_2R agonists whereas robust hypothermia was produced by the reference Aα_2R agonists but not by the reference MOR agonists. Regardless of the route of administration, MG did not produce robust antinociception or hypothermia. As with the reference MOR agonists, 7-OH-MG produced robust antinociception but did not produce significant hypothermia.

Figure 4. The rate-decreasing, antinociceptive, and hypothermic effects of reference agonists in the presence of naltrexone (NLT; opioid receptor antagonist) or yohimbine (YHM; Aα_2R antagonist). Abscissae: Vehicle and cumulative dose of reference agonist in mg/kg (i.p., log scale). Ordinates: *Top row*, percentage of mean rates of responding after repeated administration of vehicle during inter-test sessions; *middle row*, percentage of maximum possible effects in the hotplate assay; *bottom row*, changes in rectal temperature from mean baselines. Each point represents the mean ± SEM (N=4 per sex per data point). Naltrexone and yohimbine were administered i.p. immediately before each session and all reference agonists were administered i.p. 15 minutes before each 5-minute period for data collection for food-maintained behavior. Each data of compound alone (i.e. "None" in each figure key) was replotted from Figure 3. *Leftmost panels*: The effects of morphine. Morphine dose alone (filled circles) and in the presence of 3.2 mg/kg yohimbine (open squares); vehicle, 5.6, 10, 17.8, 32, and 56 mg/kg. Morphine dose in the presence of 0.032 mg/kg naltrexone (open upward triangles); vehicle, 17.8, 32, 56, 100, and 178 mg/kg. Morphine dose in the presence of 1.0 mg/kg naltrexone (open downward triangles); vehicle, 56, 100, 178, 320, and 560 mg/kg. *Second leftmost panels*: The effects of methadone. Methadone dose alone (filled circles) and in the presence of 3.2 mg/kg yohimbine (open squares); vehicle, 0.32, 0.56, 1.0, 1.78, and 3.2 mg/kg. Methadone dose in the presence of 1.0 mg/kg naltrexone (open downward triangles); vehicle, 1.0, 1.78, 3.2, 5.6, and 10 mg/kg. *Third leftmost panels*: The effects of U69,593. U69,593 dose alone (filled circles) and in the presence of 0.032 mg/kg naltrexone (open upward triangles) or

3.2 mg/kg yohimbine (open squares); vehicle, 0.56, 1.0, 1.78, 3.2, and 5.6 mg/kg. U69,593 dose in the presence of 1.0 mg/kg naltrexone (open downward triangles); vehicle, 1.78, 3.2, 5.6, 10, and 17.8 mg/kg. *Forth leftmost panels*: The effects of lofexidine. Lofexidine dose alone (filled circles) and in the presence of 1.0 mg/kg naltrexone (open downward triangles); vehicle, 0.056, 0.1, 0.178, 0.32, and 0.56 mg/kg. Lofexidine dose in the presence of 1.0 mg/kg yohimbine (diamonds); vehicle, and 0.178, 0.32, 0.56, 1.0, and 1.78 mg/kg. Lofexidine dose in the presence of 3.2 mg/kg yohimbine (open squares); vehicle, 0.56, 1.0, 1.78, 3.2, and 5.6 mg/kg. *Rightmost panels*: The effects of clonidine. Clonidine alone and in the presence of 1.0 mg/kg naltrexone (open downward triangles); vehicle, 0.0178, 0.032, 0.056, 0.1, and 0.178 mg/kg. Clonidine dose in the presence of 3.2 mg/kg yohimbine (open squares); vehicle, 0.056, 0.1, 0.178, 0.32, and 0.56 mg/kg. Each gray symbol indicates a significant difference from vehicle per corresponding cycle as shown in **Figure 3**. Note that the lower dose of naltrexone antagonized the rate-decreasing and antinociceptive effects of the reference MOR agonists. The higher dose of naltrexone antagonized the rate-decreasing and antinociceptive effects of morphine and U69,593. The lower dose of yohimbine antagonized the rate-decreasing and hypothermic effects of the reference Aα_2R agonists.

Figure 5. The rate-decreasing, antinociceptive, and hypothermic effects of MG and 7-OH-MG in the presence of naltrexone (NLT: opioid receptor antagonist) or yohimbine (YHM; Aα_2R antagonist). Abscissae: Vehicle and cumulative dose of test compound in mg/kg (i.p., log scale). Ordinates: *Top row*, percentage of mean rates of responding after repeated administration of vehicle during inter-test sessions; *middle row*, percentage of maximum possible effects in the hotplate assay; *bottom row*, changes in rectal temperature from mean baselines. Each point represents the mean ± SEM (N=4 per sex per data point). Naltrexone and yohimbine were administered i.p. immediately before each session and all other compounds were administered i.p. 15 minutes before each 5-minute period for data collection for food-maintained behavior. Each data of test compound alone (i.e. "None" in each figure key) was replotted from Figure 3. *Left panels*: The effects of MG. MG dose alone (filled circles) and in the presence of 1.0 mg/kg naltrexone (open downward triangles) or 3.2 mg/kg yohimbine (open squares); vehicle, 5.6, 10, 17.8, 32, and 56 mg/kg. *Right panels*: The effects of 7-OH-MG. 7-OH-MG dose alone (filled circles) and in the presence of 3.2 mg/kg yohimbine (open squares); vehicle,

0.32, 1.0, 3.2, 10, and 32 mg/kg. 7-OH-MG dose in the presence of .032 mg/kg naltrexone (open upward triangles); vehicle, 1.0, 3.2, 10, 32, and 56 mg/kg. Each gray symbol indicates a significant difference from vehicle per corresponding cycle as shown in **Figure 3**. Note that each high dose of naltrexone and yohimbine did not significantly antagonize the rate-decreasing effects of MG. The lower dose of naltrexone antagonized the rate-decreasing and antinociceptive effects of 7-OH-MG.

Figure 6. The rate-decreasing, antinociceptive, and hypothermic effects of reference agonists in the presence of MG and 7-OH-MG. Abscissae: Vehicle and cumulative dose of reference agonist in mg/kg (i.p., log scale). Ordinates: *Top row*, percentage of mean rates of responding after repeated administration of vehicle during inter-test sessions; *middle row*, percentage of maximum possible effects in the hotplate assay; *bottom row*, changes in rectal temperature from mean baselines. Each point represents the mean ± SEM (N=4 per sex per data point). MG and 7-OH-MG were administered i.p. immediately before each session and all reference agonists were administered i.p. 15 minutes before each 5-minute period for data collection for food-maintained behavior. Each data of reference agonists alone (i.e. "None" in each figure key) was replotted from **Figure 3**. *Leftmost panels*: The effects of morphine. Morphine dose alone (filled circles) and in the presence of 17.8 mg/kg MG (open squares) or 0.32 mg/kg 7-OH-MG (open diamonds); vehicle, 5.6, 10, 17.8, 32, and 56 mg/kg. *Second leftmost panels*: The effects of methadone. Methadone dose alone (filled circles) and in the presence of 17.8 mg/kg MG (open squares) or 0.32 mg/kg 7-OH-MG (open diamonds); vehicle, 0.32, 0.56, 1.0, 1.78, and 3.2 mg/kg. *Third leftmost panels*: The effects of U69,593. U69,593 dose alone (filled circles) and in the presence of 17.8 mg/kg MG (open squares) or 0.32 mg/kg 7-OH-MG (open diamonds); vehicle, 0.56, 1.0, 1.78, 3.2, and 5.6 mg/kg. *Forth leftmost panels*: The effects of lofexidine. Lofexidine dose alone (filled circles) and in the presence of 0.32 mg/kg 7-OH-MG (open diamonds); vehicle, 0.056, 0.1, 0.178, 0.32, and 0.56 mg/kg. Lofexidine dose in the presence of 17.8 mg/kg MG (open squares); vehicle, 0.0178, 0.032, 0.056, 0.1, and 0.178 mg/kg. *Rightmost panels*: The effects of clonidine. Clonidine alone and in the presence of 0.32 mg/kg 7-OH-MG (open diamonds); vehicle, 0.0178, 0.032, 0.056, 0.1, and 0.178 mg/kg. Clonidine dose in the presence of 17.8 mg/kg MG (open squares); vehicle, 0.0056, 0.01, 0.0178, 0.032, and 0.056 mg/kg. Each gray symbol indicates a significant difference from vehicle per corresponding cycle as shown in **Figure 3**.

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Note that MG potentiated the rate-decreasing and hypothermic effects of the reference $A\alpha_2R$ agonists. In the presence of MG and 7-OH-MG, the reference $A\alpha_2R$ agonists also produced relatively robust antinociception.

Figure 7. Isobolographic analysis of reference $A\alpha_2R$ agonists combined with MOR or KOR reference agonists. Ordinates, ED_{50} values of morphine (*left panels*), methadone (*middle panels*), and U69,593 (*right panels*) in mg/kg. Abscissae, ED_{50} values of lofexidine (*upper panels*) and clonidine (*lower panels*) in mg/kg. Each point represents the ED_{50} value and error bars represent 95% CIs. The points at which the line of additivity crosses the ordinates and abscissae represent the ED_{50} values of each compound alone. The line of additivity (dashed line) represents combinations of doses that would be predicted to produce a 50% effect if the compounds were strictly dose-additive. The vertical and horizontal lines around each data point represent the 95% CIs. * Indicates at least $p < 0.05$ difference between Zmix and Zadd for a respective dose combination, denoting supra-additivity.

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Table 1 Inhibition of binding of the radioligands labeling $A\alpha_2R$ and opioid receptor subtypes. Values are Ki values for displacement of the radioligands (see Table S1). Values in parentheses are 95% CIs unless noted. Values listed from previous studies were also added as reference.

Compound	$A\alpha_{2A}R$ K_i Value (nM)	$A\alpha_{2C}R$ K_i Value (nM)	DOR K_i Value (nM)	KOR K_i Value (nM)	MOR K_i Value (nM)	$A\alpha_{2C}$ /$A\alpha_{2A}$	$A\alpha_{2A}$/MOR	$A\alpha_{2C}$/MOR
Clonidine	5.97 (3.66, 10.4)	60.8 (33.7, 115)	No inhibition up to 10 μM	No inhibition up to 10 μM	No inhibition up to 10 μM	10.2	NA	NA
7-OH-MG	No inhibition up to 10 μM	No inhibition up to 10 μM	243 (168, 355)	220 (162, 302)	77.9 (45.8, 152)	NA	NA	NA
Lofexidine	1.21 (0.60, 2.43)	7.62 (3.96, 14.8)	No inhibition up to 10 μM	No inhibition up to 10 μM	No inhibition up to 10 μM	6.30	NA	NA
Methadone	No inhibition up to 10 μM	No inhibition up to 10 μM	No inhibition up to 10 μM	481 (294, 816)	6.61 (5.27, 8.32)	NA	NA	NA
MG	4,420 (2,720,	4,040 (1,880,	6,800 (2,980,	1,700 (1,090,	709 (451, 1,130)[a]	0.914	6.23	5.70

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	7,670)[a] 4,720 (SEM: 120)[b] 2.3 μM[c]	6,820)[a] 2,320 (SEM: 140)[b] 3.5 μM[c]	15,900)[a]	2,710)[a]				
Morphine	No inhibition up to 10 μM	No inhibition up to 10 μM	250 (177, 346)[a]	40.4 (23.7, 70.9)[a]	4.19 (2.03, 11.1)[a]	NA	NA	NA
Naltrexone	No inhibition up to 10 μM	No inhibition up to 10 μM	37.2 (26.3, 53.0)[a]	1.19 (0.803, 1.79)[a]	1.84 (1.14, 3.03)[a]	NA	NA	NA
U69,593	No inhibition up to 10 μM	No inhibition up to 10 μM	6,700 (2,160, 28,000)[a]	1.62[a] (1.02, 2.64)[a]	3,180 (1,050, 11,600)[a]	NA	NA	NA
Yohimbine	8.24 (5.40, 12.8)	7.77 (4.76, 12.8)	No inhibition up to 10 μM	No inhibition up to 10 μM	No inhibition up to 10 μM	0.943	NA	NA

Ki: Inhibition constant.

NA: Not applicable

[a] Human recombinant CHO cells using [^{3}H]RX821002 conducted at Eurofins Cerep (Celle l'Evescault, France) (Obeng et al., 2020).

[b] Binding at human opioid receptor cell lines (Obeng et al., 2021b).

[c] Binding at adrenergic receptors ($A\alpha_{2A}$ and $A\alpha_{2C}$) conducted at the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH, PDSP) (Ellis et al., 2020).

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Table 2 ED_{50} and E_{-2C° values in mg/kg for the rate-decreasing, antinociceptive, hypothermic effects of various compounds as shown in **Figures 3-6, S2-4**. The sample sizes are described in each figure legend. Each value is a combination of females and males unless otherwise noted. Potency ratios (SEMs) are calculated by dividing the ED_{50} or E_{-2C° values for producing the antinociceptive or hypothermic effects, respectively, by the ED_{50} values for producing the rate-decreasing effects. Values in parentheses are 95% CIs. Significant differences are bold.

Morphine Dose					
Combination	ED_{50} or E_{-2C° (SEM)			Potency Ratio	
	Decrease in Response Rate (ED_{50})	Antinociception (ED_{50})	Hypothermia (E_{-2C°)	Antinociception / Decrease in Response Rate	Hypothermia / Decrease in Response Rate
Morphine Alone	9.81 (7.32, 12.30)	39.30 (37.18, 41.43)	Not Applicable	**4.00 (3.02, 5.66)**	Not Applicable
Morphine + 0.032 mg/kg Naltrexone	43.8 (41.6, 46.0)	210 (188, 232)	Not Applicable	**4.79 (4.09, 5.58)**	Not Applicable
Morphine + 1.0 mg/kg Naltrexone	309 (257, 361)	Not Applicable	Not Applicable	Not Applicable	Not Applicable
Morphine + 3.2 mg/kg Yohimbine	13.5 (11.1, 15.9)	24.3 (19.0, 29.6)	Not Applicable	**1.80 (1.20, 2.67)**	Not Applicable
Morphine + 17.8 mg/kg MG	9.29 (6.55, 12.03)	35.9 (33.8, 38.0)	Not Applicable	**3.86 (2.81, 5.80)**	Not Applicable
Morphine + 0.32 mg/kg	19.5 (15.8, 23.2)	33.1 (29.6, 36.6)	Not	**1.70 (1.28, 2.32)**	Not

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7-OH-MG			Applicable		Applicable
Methadone Dose					
Combination	ED_{50} or $E_{-2C^{\circ}}$ (SEM)			Potency Ratio	
	Decrease in Response Rate (ED_{50})	Antinociception (ED_{50})	Hypothermia ($E_{-2C^{\circ}}$)	Antinociception / Decrease in Response Rate	Hypothermia / Decrease in Response Rate
Methadone Alone	0.70 (0.48, 0.92)	2.22 (1.74, 2.70)	Not Applicable	**3.17 (1.89, 5.63)**	Not Applicable
Methadone + 0.032 mg/kg Naltrexone	2.87 (2.65, 3.09)	25.3 (24.3, 26.3)	Not Applicable	**8.81 (7.86, 9.92)**	Not Applicable
Methadone + 3.2 mg/kg Yohimbine	1.19 (1.00, 1.40)	2.28 (1.80, 2.76)	Not Applicable	**1.91 (1.29, 2.76)**	Not Applicable
Methadone + 17.8 mg/kg MG	1.04 (0.92, 1.16)	2.25 (2.05, 2.45)	Not Applicable	**2.16 (1.77, 2.66)**	Not Applicable
Methadone + 0.32 mg/kg 7-OH-MG	1.16 (0.86, 1.50)	1.93 (1.86, 2.00)	Not Applicable	**1.66 (1.24, 2.33)**	Not Applicable
U69,593 Dose					
Combination	ED_{50} or $E_{-2C^{\circ}}$ (SEM)			Potency Ratio	
	Decrease in Response Rate (ED_{50})	Antinociception (ED_{50})	Hypothermia ($E_{-2C^{\circ}}$)	Antinociception / Decrease in Response Rate	Hypothermia / Decrease in Response Rate
U69,593 Alone	2.17 (1.70, 2.65)	3.17 (2.39, 3.95)	Not	1.46 (0.90, 2.32)	Not

			Applicable		Applicable
U69,593 + 0.032 mg/kg Naltrexone	Not Applicable	1.86 (1.52, 2.20)	Not Applicable	Not Applicable	Not Applicable
U69,593 + 1.0 mg/kg Naltrexone	14.58 (11.87, 17.29)	49.07 (47.0, 51.20)	Not Applicable	**3.36 (2.72, 4.31)**	Not Applicable
U69,593 + 3.2 mg/kg Yohimbine	2.28 (1.80, 2.80)	2.62 (1.84, 3.40)	Not Applicable	1.15 (0.657, 1.89)	Not Applicable
U69,593 + 17.8 mg/kg MG	3.10 (2.74, 3.46)	4.66 (4.15, 5.17)	Not Applicable	**1.50 (1.19, 1.89)**	Not Applicable
U69,593 + 0.32 mg/kg 7-OH-MG	5.61 (4.67, 6.55)	16.0 (15.0, 16.9)	Not Applicable	2.85 (2.29, 3.62)	Not Applicable
Lofexidine Dose					
Combination	ED_{50} or $E_{-2C°}$ (SEM)			Potency Ratio	
	Decrease in Response Rate (ED_{50})	Antinociception (ED_{50})	Hypothermia ($E_{-2C°}$)	Antinociception / Decrease in Response Rate	Hypothermia / Decrease in Response Rate
Lofexidine Alone	0.153 (0.121, 0.185)	Not Applicable	0.294 (0.267, 0.321)	Not Applicable	**1.92 (1.44, 2.65)**
Lofexidine + 1.0 mg/kg Naltrexone	0.107 (0.085, 0.129)	Not Applicable	0.395 (0.332, 0.458)	Not Applicable	**3.69 (2.57, 5.39)**
Lofexidine + 1.0 mg/kg Yohimbine	0.788 (0.683, 0.893)	Not Applicable	1.06 (0.887, 1.23)	Not Applicable	1.35 (0.993, 1.80)

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Lofexidine + 3.2 mg/kg Yohimbine	1.89 (1.60, 2.18)	Not Applicable	3.69 (2.84, 4.54)	Not Applicable	**1.95 (1.30, 2.84)**
Lofexidine + 17.8 mg/kg MG	0.019 (0.014, 0.024)	0.168 (0.161, 0.175)	0.037 (0.027, 0.046)	**8.84 (6.71, 12.5)**	**1.95 (1.13, 3.29)**
Lofexidine + 0.32 mg/kg 7-OH-MG	Not Applicable	0.472 (0.457, 0.487)	0.208 (0.181, 0.235)	Not Applicable	Not Applicable
Clonidine Dose					
Combination	ED_{50} or $E_{-2C°}$ (SEM)			Potency Ratio	
	Decrease in Response Rate (ED_{50})	Antinociception (ED_{50})	Hypothermia ($E_{-2C°}$)	Antinociception / Decrease in Response Rate	Hypothermia / Decrease in Response Rate
Clonidine Alone	0.048 (0.038, 0.058)	Not Applicable	0.094 (0.088, 0.100)	Not Applicable	**1.96 (1.52, 2.63)**
Clonidine + 1.0 mg/kg Naltrexone	0.054 (0.044, 0.064)	Not Applicable	0.105 (0.087, 0.123)	Not Applicable	1.94 (1.36, 2.80)
Clonidine + 1.0 mg/kg Yohimbine	0.186 (0.159, 0.213)	Not Applicable	0.544 (0.474, 0.614)	Not Applicable	2.92 (2.23, 3.86)
Clonidine + 17.8 mg/kg MG	Not Applicable (no more than 50% data point)	0.042 (0.039, 0.186)	0.0633 (0.0501, 0.045)	Not Applicable	Not Applicable
Clonidine + 0.32 mg/kg 7-OH-MG	Not Applicable (no more than 50% data point)	Not Applicable (up to 47.5% MPE)	0.093 (0.089, 0.097)	Not Applicable	Not Applicable

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MG Dose					
Combination	ED_{50} or $E_{-2C^{\circ}}$ (SEM)			Potency Ratio	
	Decrease in Response Rate (ED_{50})	Antinociception (ED_{50})	Hypothermia ($E_{-2C^{\circ}}$)	Antinociception / Decrease in Response Rate	Hypothermia / Decrease in Response Rate
MG Alone (i.p.)	27.2 (21.0, 33.4)	Not Applicable	Not Applicable	Not Applicable	Not Applicable
MG (i.p.) + 1.0 mg/kg Naltrexone	33.8 (22.7, 45.0)	Not Applicable	Not Applicable	Not Applicable	Not Applicable
MG (i.p.) + 3.2 mg/kg Yohimbine	32.0 (27.0, 37.0)	Not Applicable	Not Applicable	Not Applicable	Not Applicable
MG Alone (p.o.)	89.3 (69.8, 108)	Not Applicable	Not Applicable	Not Applicable	Not Applicable
MG Alone (s.c.)	161 (118, 204)	Not Applicable	Not Applicable	Not Applicable	Not Applicable
7-OH-MG Dose					
Combination	ED_{50} or $E_{-2C^{\circ}}$ (SEM)			Potency Ratio	
	Decrease in Response Rate (ED_{50})	Antinociception (ED_{50})	Hypothermia ($E_{-2C^{\circ}}$)	Antinociception / Decrease in Response Rate	Hypothermia / Decrease in Response Rate
7-OH-MG Alone	1.82 (1.22, 2.42)	9.13 (7.41, 10.9)	Not Applicable	**5.02 (3.06, 8.93)**	Not Applicable

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7-OH-MG + 0.032 mg/kg Naltrexone	17.5 (14.4, 20.7)	41.8 (38.2, 45.5)	Not Applicable	**2.39 (1.85, 3.16)**	Not Applicable
7-OH-MG + 3.2 mg/kg Yohimbine	3.07 (2.53, 3.61)	15.7 (14.1, 17.3)	Not Applicable	**5.11 (3.91, 6.84)**	Not Applicable

Table 3 Cumulative doses of test compounds (mg/kg) studied in compound mixtures. Values in parentheses are S.E.M.

1 Morphine : 1 Lofexidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Morphine	Vehicle	1.79 (0.447)	3.19 (0.795)	5.69 (1.42)	10.1 (2.52)	18.0 (4.48)
Lofexidine	Vehicle	0.0196 (0.00433)	0.0348 (0.00771)	0.0620 (0.0137)	0.110 (0.0244)	0.196 (0.0435)
1 Morphine : 2 Lofexidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Morphine	Vehicle	0.897 (0.223)	1.60 (0.398)	2.84 (0.708)	5.06 (1.26)	9.01 (2.24)
Lofexidine	Vehicle	0.0293 (0.00650)	0.0522 (0.0116)	0.0930 (0.0206)	0.165 (0.0367)	0.295 (0.0652)
3 Morphine : 1 Lofexidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Morphine	Vehicle	2.69 (0.670)	4.79 (1.19)	8.53 (2.12)	15.2 (3.78)	27.0 (6.73)
Lofexidine	Vehicle	0.00978 (0.00217)	0.0174 (0.00386)	0.0310 (0.00686)	0.0552 (0.0122)	0.0982 (0.0217)
2 Morphine : 1 Clonidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Morphine	Vehicle	1.79 (0.447)	3.19 (0.795)	5.69 (1.42)	10.1 (2.52)	18.0 (4.48)
Clonidine	Vehicle	0.00379 (0.000994)	0.00675 (0.00177)	0.0120 (0.00315)	0.0214 (0.00561)	0.0381 (0.00998)

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1 Morphine : 2 Clonidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Morphine	Vehicle	0.897 (0.223)	1.60 (0.398)	2.84 (0.708)	5.06 (1.26)	9.01 (2.24)
Clonidine	Vehicle	0.00569 (0.00149)	0.0101 (0.00266)	0.0180 (0.00473)	0.0321 (0.00841)	0.0571 (0.0150)
3 Morphine : 1 Clonidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Morphine	Vehicle	2.69 (0.670)	4.79 (1.19)	8.53 (2.12)	15.2 (3.78)	27.0 (6.73)
Clonidine	Vehicle	0.00190 (0.000497)	0.00338 (0.000885)	0.00601 (0.00158)	0.0107 (0.00280)	0.0190 (0.00499)
1 Methadone : 1 Lofexidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Methadone	Vehicle	0.144 (0.0429)	0.257 (0.0764)	0.457 (0.136)	0.813 (0.242)	1.45 (0.431)
Lofexidine	Vehicle	0.0196 (0.00433)	0.0348 (0.00771)	0.0620 (0.0137)	0.110 (0.0244)	0.196 (0.0435)
1 Methadone : 2 Lofexidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Methadone	Vehicle	0.0721 (0.0215)	0.128 (0.0382)	0.228 (0.0680)	0.407 (0.121)	0.724 (0.215)
Lofexidine	Vehicle	0.0293 (0.00650)	0.0522 (0.0166)	0.0930 (0.0206)	0.165 (0.0367)	0.295 (0.0652)
3 Methadone : 1 Lofexidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Methadone	Vehicle	0.216 (0.0644)	0.385 (0.115)	0.685 (0.204)	1.22 (0.363)	2.17 (0.646)
Lofexidine	Vehicle	0.00978 (0.00217)	0.0174 (0.00386)	0.0310 (0.00686)	0.0552 (0.0122)	0.0982 (0.0217)
2 Methadone : 1 Clonidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6

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Methadone	Vehicle	0.144 (0.0429)	0.257 (0.0764)	0.457 (0.136)	0.813 (0.242)	1.45 (0.431)
Clonidine	Vehicle	0.00379 (0.000994)	0.00675 (0.00177)	0.0120 (0.00315)	0.0214 (0.00561)	0.0381 (0.00998)
1 Methadone : 1 Clonidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Methadone	Vehicle	0.0721 (0.0215)	0.128 (0.0382)	0.228 (0.0680)	0.406 (0.121)	0.724 (0.215)
Clonidine	Vehicle	0.00569 (0.00149)	0.0101 (0.00266)	0.0180 (0.00473)	0.0321 (0.00841)	0.0571 (0.0150)
4 Methadone : 1 Clonidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Methadone	Vehicle	0.216 (0.0644)	0.385 (0.115)	0.685 (0.204)	1.22 (0.363)	2.17 (0.646)
Clonidine	Vehicle	0.00190 (0.000467)	0.00338 (0.000885)	0.00601 (0.00158)	0.0107 (0.00280)	0.0190 (0.00499)
1 U69,593 : 1 Lofexidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
U69,593	Vehicle	0.346 (0.0471)	0.616 (0.0839)	1.10 (0.149)	1.95 (0.266)	3.47 (0.473)
Lofexidine	Vehicle	0.0177 (0.00346)	0.0316 (0.00616)	0.0562 (0.0110)	0.100 (0.0195)	0.178 (0.0348)
1 U69,593 : 2 Lofexidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
U69,593	Vehicle	0.173 (0.0236)	0.308 (0.0420)	0.548 (0.0747)	0.975 (0.133)	1.74 (0.237)
Lofexidine	Vehicle	0.0339 (0.00757)	0.0603 (0.135)	0.107 (0.0240)	0.191 (0.0427)	0.340 (0.760)
2 U69,593 : 1 Lofexidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
U69,593	Vehicle	0.519 (0.0707)	0.923 (0.126)	1.64 (0.224)	2.93 (0.399)	5.21 (0.710)

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Lofexidine	Vehicle	0.0113 (0.00252)	0.0201 (0.00449)	0.0358 (0.00800)	0.0637 (0.0142)	0.113 (0.0253)
2 U69,593 : 1 Clonidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
U69,593	Vehicle	0.346 (0.0471)	0.616 (0.0839)	1.10 (0.149)	1.95 (0.266)	3.47 (0.473)
Clonidine	Vehicle	0.00382 (0.000956)	0.00680 (0.00170)	0.0121 (0.00303)	0.0215 (0.00539)	0.0384 (0.00960)
1 U69,593 : 2 Clonidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
U69,593	Vehicle	0.173 (0.0236)	0.308 (0.0420)	0.548 (0.0747)	0.975 (0.133)	1.74 (0.237)
Clonidine	Vehicle	0.00761 (0.00215)	0.0135 (0.00383)	0.0241 (0.00681)	0.0429 (0.0121)	0.0764 (0.0216)
3 U69,593 : 1 Clonidine						
Compound	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
U69,593	Vehicle	0.519 (0.0707)	0.923 (0.126)	1.64 (0.224)	2.93 (0.399)	5.21 (0.710)
Clonidine	Vehicle	0.00254 (0.000717)	0.00451 (0.00128)	0.00803 (0.00227)	0.0143 (0.00404)	0.0255 (0.00720)

Table 4 Theoretical Zadd (mg/kg), Experimental Zmix (mg/kg), their confidence intervals, and observed interactive effects of studied compound mixtures.

1 Morphine : 1 Lofexidine		
Zadd	Zmix	Interactive Effect
9.13 (6.55 - 11.71)	2.88 (1.94 - 3.81)	Supra-Additive
1 Morphine : 2 Lofexidine		
Zadd	Zmix	Interactive Effect
9.00 (6.45 – 11.54)	1.42 (0.932 – 1.91)	Supra-Additive

3 Morphine : 1 Lofexidine		
Zadd	Zmix	Interactive Effect
9.22 (3.14 – 15.31)	4.00 (2.23 – 6.23)	Additive
2 Morphine : 1 Clonidine		
Zadd	Zmix	Interactive Effect
9.26 (6.64 – 11.84)	2.90 (1.15 – 4.66)	Supra-Additive
1 Morphine : 2 Clonidine		
Zadd	Zmix	Interactive Effect
9.24 (6.63– 11.86)	1.57 (1.06 – 2.08)	Supra-Additive
3 Morphine : 1 Clonidine		
Zadd	Zmix	Interactive Effect
9.26 (6.65 – 11.88)	5.73 (3.50 – 7.95)	Additive
1 Methadone : 1 Lofexidine		
Zadd	Zmix	Interactive Effect
0.604 (0.541 – 0.668)	0.514 (0.084 – 0.944)	Additive
1 Methadone : 2 Lofexidine		
Zadd	Zmix	Interactive Effect
0.540 (0.159 - 0.922)	0.237 (0.170 – 0.304)	Additive
3 Methadone : 1 Lofexidine		
Zadd	Zmix	Interactive Effect
0.660 (0.279 – 1.04)	0.767 (0.488 – 1.05)	Additive
2 Methadone : 1 Clonidine		
Zadd	Zmix	Interactive Effect
0.683 (0.608 – 0.757)	0.280 (0.137 – 0.424)	Supra-Additive
1 Methadone : 1 Clonidine		

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Zadd	Zmix	Interactive Effect
0.672 (0.415 – 0.930)	0.183 (0.0261 – 0.340)	Supra-Additive
4 Methadone : 1 Clonidine		
Zadd	Zmix	Interactive Effect
0.688 (0.590 – 0.786)	0.680 (0.224 – 1.14)	Additive
1 U69,593 : 1 Lofexidine		
Zadd	Zmix	Interactive Effect
2.033 (1.71 – 2.35)	1.01 (0.907 – 1.108)	Supra-Additive
1 U69,593 : 2 Lofexidine		
Zadd	Zmix	Interactive Effect
1.91 (1.25 – 2.57)	0.484 (0.395 – 0.573)	Supra-Additive
2 U69,593 : 1 Lofexidine		
Zadd	Zmix	Interactive Effect
2.102 (1.73 – 2.47)	1.23 (0.903 – 1.56)	Supra-Additive
2 U69,593 : 1 Clonidine		
Zadd	Zmix	Interactive Effect
2.16 (1.82 – 2.50)	0.735 (0.189 – 1.28)	Supra-Additive
1 U69,593 : 2 Clonidine		
Zadd	Zmix	Interactive Effect
2.12 (1.73 – 2.51)	0.567 (0.462 – 0.672)	Supra-Additive
3 U69,593 : 1 Clonidine		
Zadd	Zmix	Interactive Effect
2.17 (1.82 – 2.51)	1.63 (1.38 – 1.87)	Additive

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Figure 1

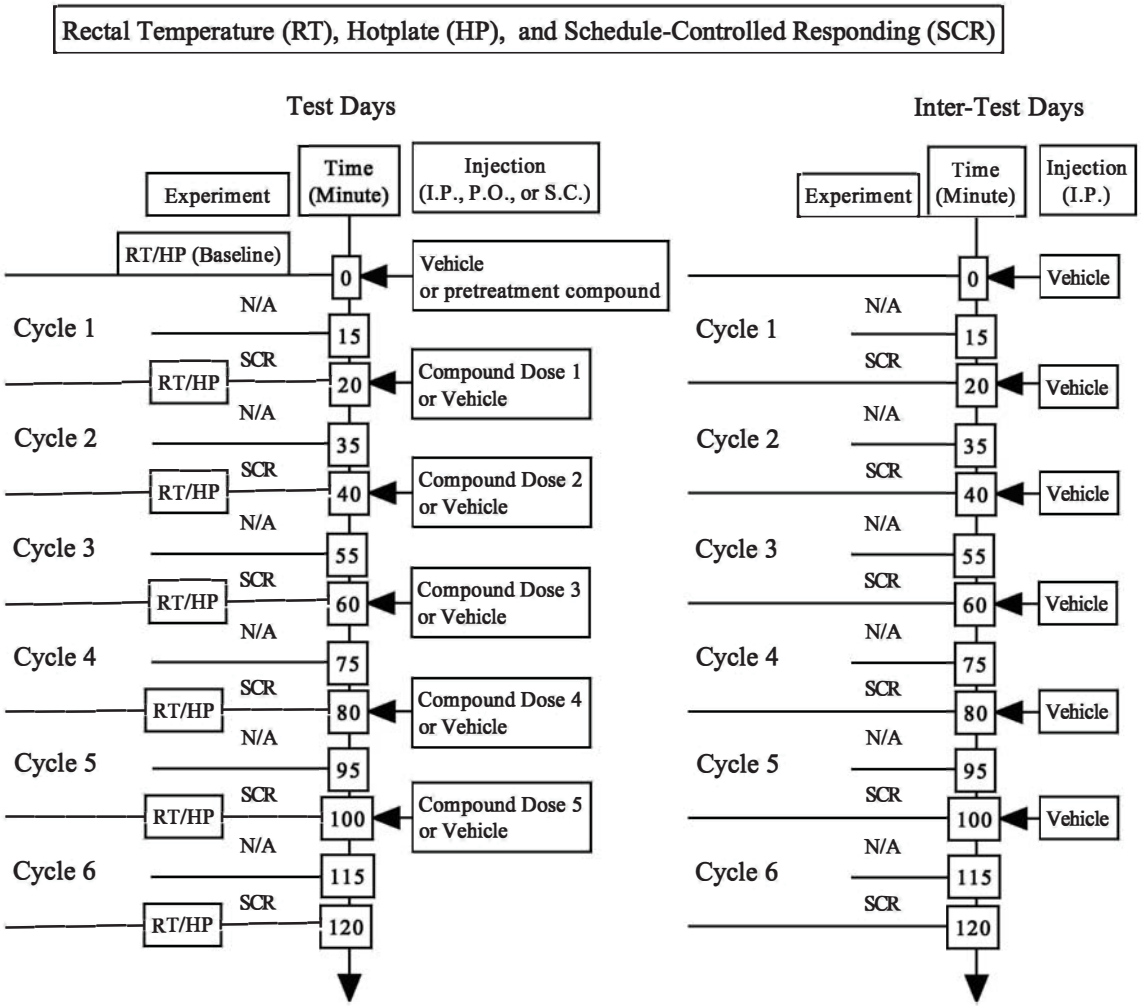

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Figure 2

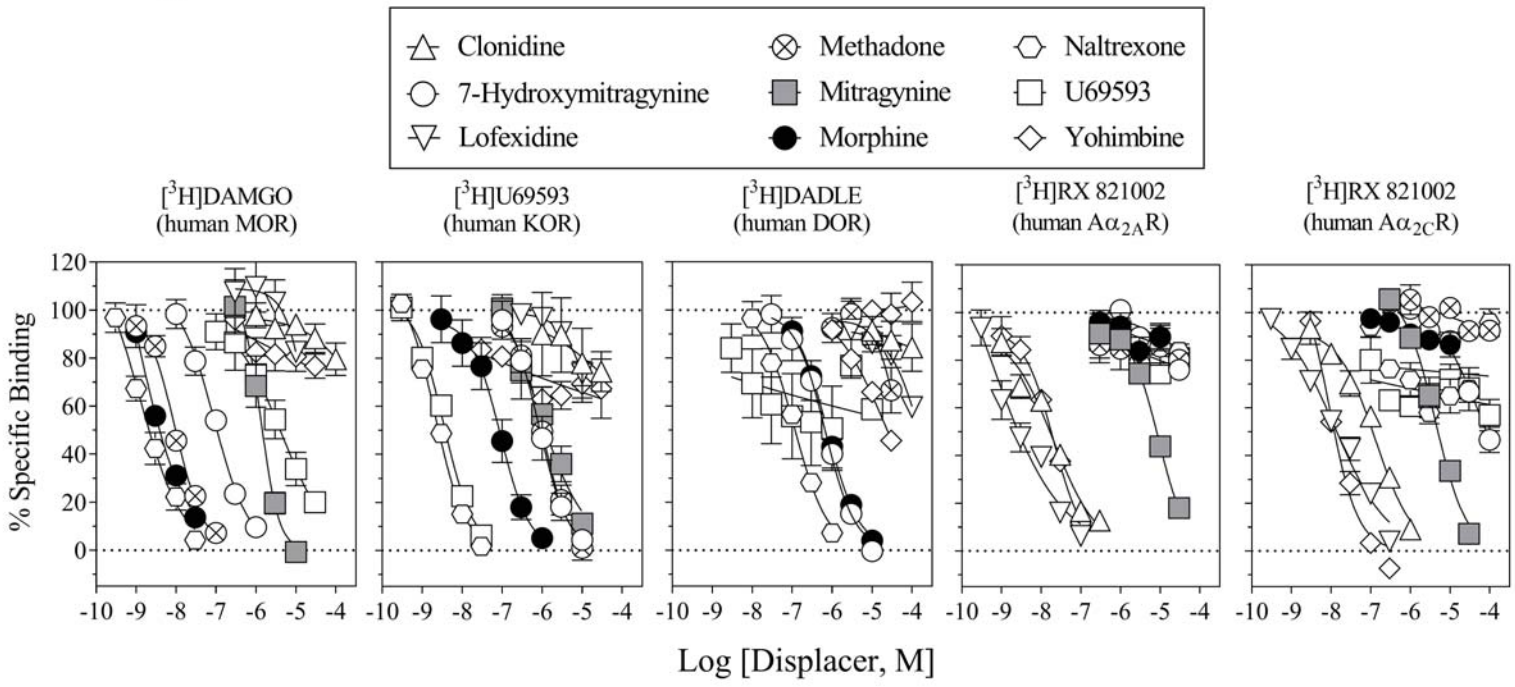

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Figure 3

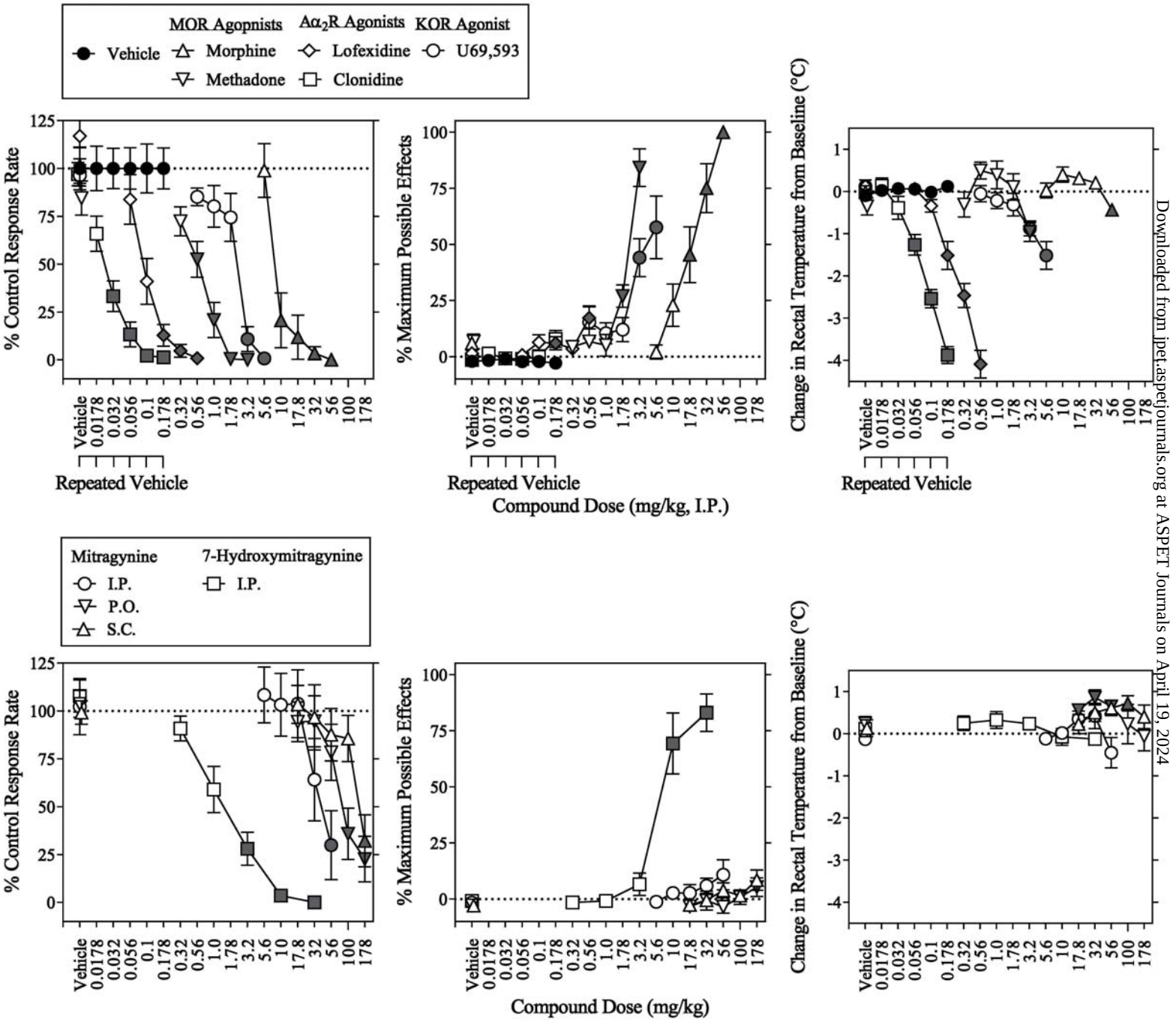

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Figure 4

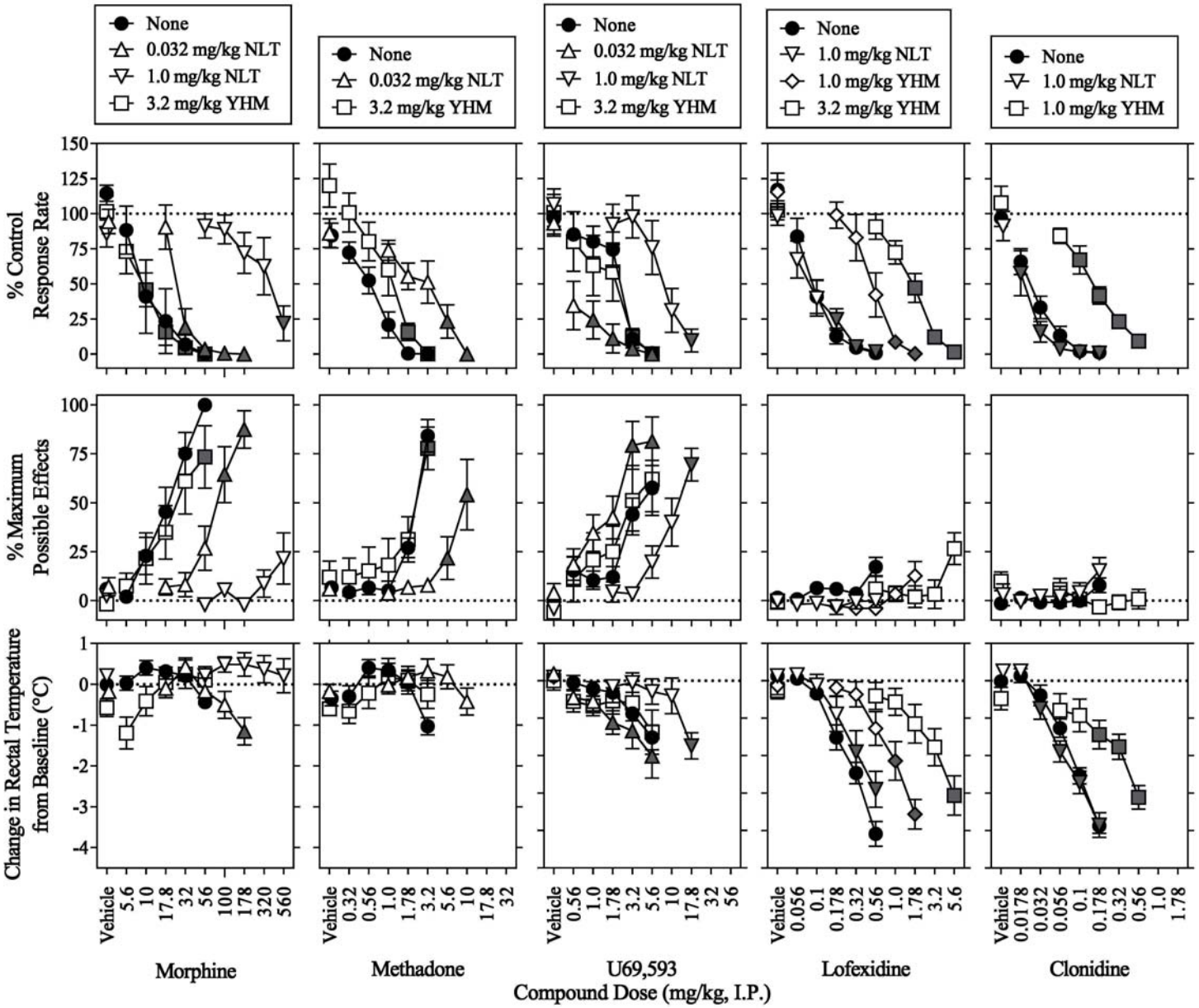

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Figure 5

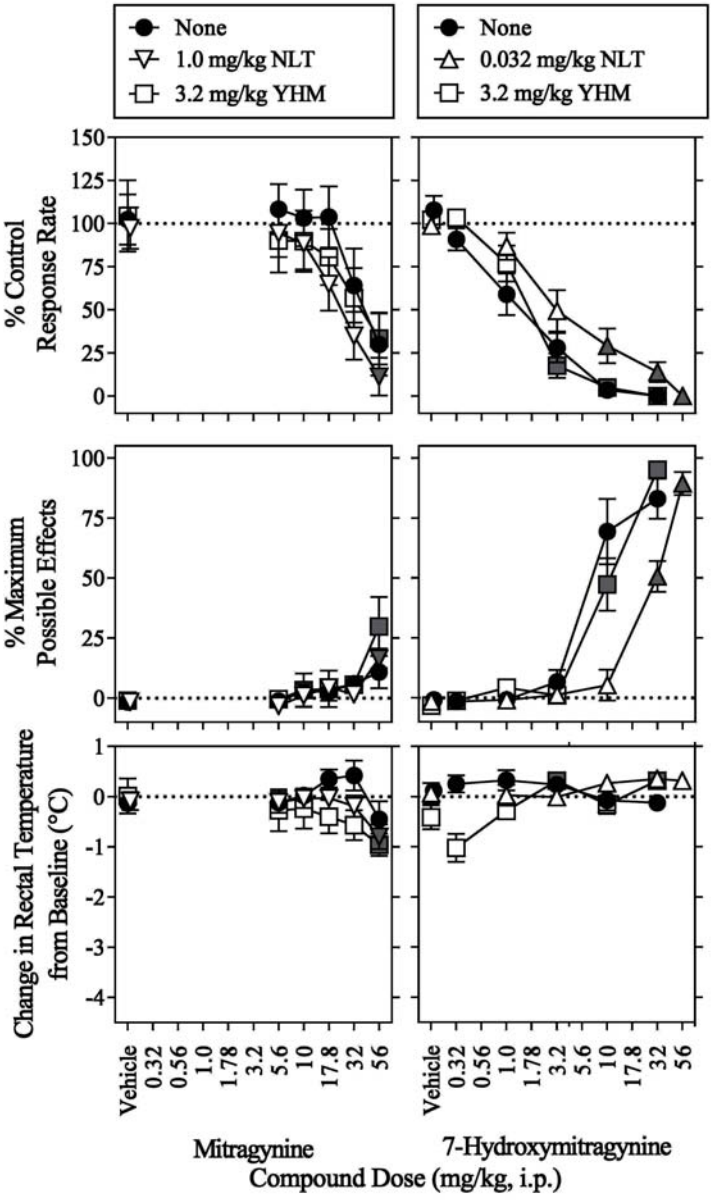

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Figure 6

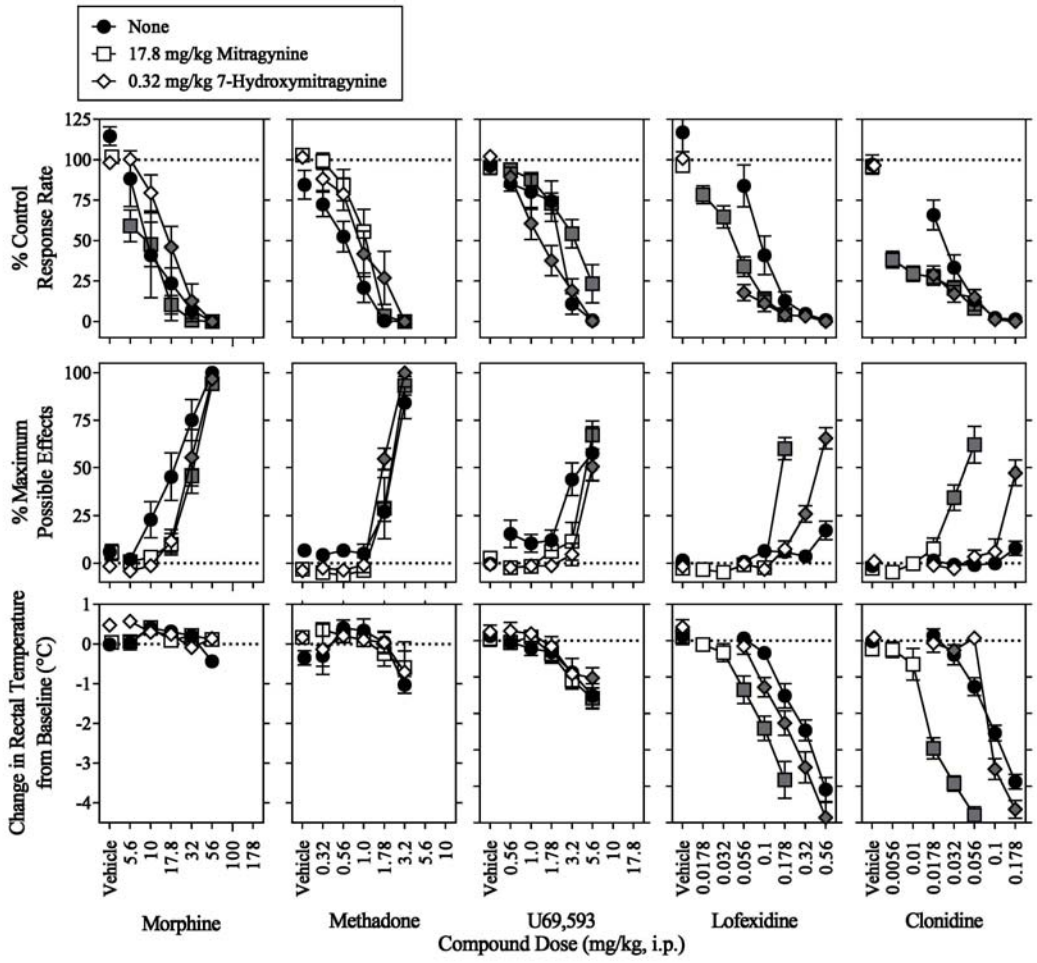

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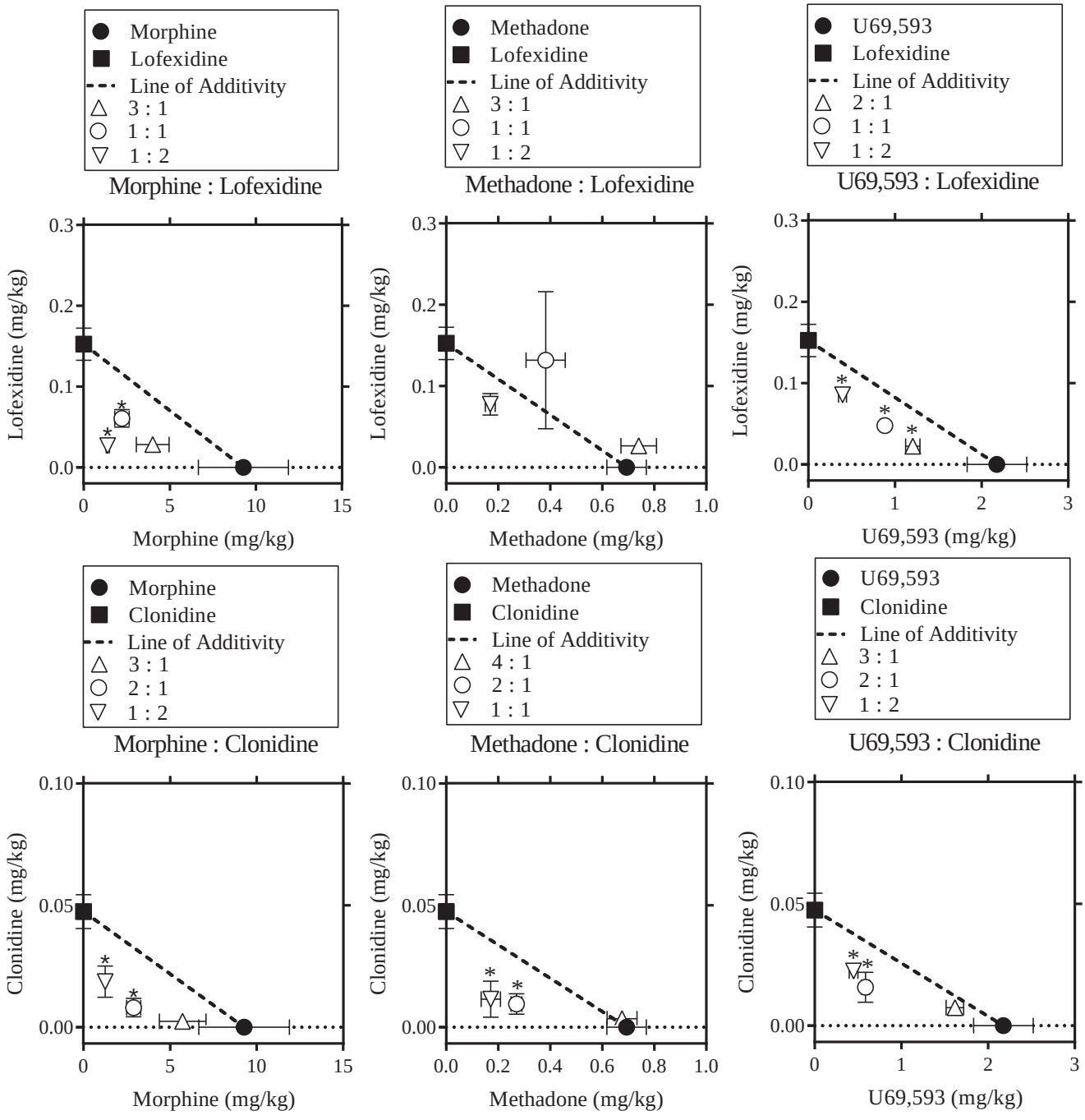
Figure 7



Supplemental Materials

Interactive Effects of μ-Opioid and Adrenergic-Alpha$_2$ Receptor Agonists Using a Schedule-Controlled Responding Assay in Rats: Comparison with Mitragynine, the Primary Kratom Alkaloid, and its Metabolite 7-Hydroxymitragynine

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RESULTS

Repeated Injections of Vehicle. The mean baseline values for rates of lever responding, hotplate latency and rectal temperature were 1.10 (SEM: ±0.11) responses/second, 10.1 (0.67) seconds and 38.0 (0.10) °C. There was no significant effect of sex on each baseline value ($F_{1,6}$ values ≤ 2.59; P values ≥ 0.159). Following repeated injections of vehicle, each variable was statistically stable across cycles (i.e., did not differ significantly) regardless of the routes of administration (Figure S1, left panels, and Table S2). There was no statistically significant difference in sex or interaction of sex with cycle regardless of the route of administration (Table S2). Finally, there was no significant difference in sex or interaction of sex with cycle, or first or second assessment for repeated i.p. injection of vehicle (Figure S1, right panels, and Table S3).

Reference MOR Agonists Alone. Morphine dose-dependently and significantly decreased response rates and rectal temperature, and increased antinociception (Figure 3, upper panels, upward triangles; Table S4). There was no significant effect of sex or interaction of sex with morphine dose on rates of responding, antinociception, and changes in rectal temperature (Table S4). Stability of the dose-effect functions of morphine was assessed following completion of all other studies. Relative to the first dose-effect assessment of morphine, there was no significant potency or slope across the rates of responding, antinociception, or rectal temperature (Figure S2; Tables S4-S5). Thus, there was no development of tolerance to the activity of morphine.

As with morphine, methadone significantly decreased response rates and rectal temperature, and produced antinociception (Figure 3, upper panels, downward triangles; Table S4). There was no significant effect of sex or interaction of sex with methadone dose (Table S4).

Reference KOR Agonist Alone. U69,593 significantly decreased response rates and rectal temperature, and significantly produced antinociception (Figure 3, upper panels, circles; Table S6). There was no significant effect of sex or interaction of sex with U69,593 dose on the rates of responding whereas significant effects of sex were found regarding antinociception and changes in rectal temperature (Table S6). In addition, there was a significant effect of interaction of sex with U69,593 dose on hotplate antinociception (Table S6). The significant antinociceptive effects of U69,593 were observed in only females (Table S6). U69,593 was more potent to produce hypothermia in females than in males (Table S6).

Reference Aα_2R Agonists Alone. Lofexidine significantly decreased response rates and rectal temperature, and significantly produced antinociception; the antinociceptive effects of lofexidine reached statistical significance but the maximum effects of lofexidine were at least 4-fold less than those of reference MOR agonists (Figure 3, upper panels, diamonds; Table S7). In contrast, as compared to the reference MOR agonists, the hypothermic effects of lofexidine were significantly greater, e.g., 4.1°C decrease in rectal temperature at 0.56 mg/kg (Figure 3). There was no significant effect of sex or interaction of sex with lofexidine dose on rates of responding, antinociception or changes in rectal temperature (Table S7).

Clonidine significantly decreased response rates and rectal temperature; however, no statistically significant change in antinociception was obtained (Figure 3, upper panels, squares; Table S7). There was no significant effect of sex or interaction of sex with clonidine dose (Table S7).

MG and 7-OH-MG Alone. When administered i.p., MG significantly decreased response rates; however, no significant effect was obtained on antinociception or rectal temperature (Figure 3, lower panels, circles; Table S8). There was no significant effect of sex or interaction of sex with MG dose on rates of responding, antinociception, and changes in rectal temperature (Table S8). The lack of the antinociceptive and hypothermic effects of MG in the present study (Figure 3; Table S8) was not expected due to the literature showing the sensitivity of MG to antagonists at the opioid receptor and Aα_2R (Foss et al., 2020; Obeng et al., 2021b) and conversion of MG to MOR active metabolites (Kruegel et al., 2019; Kamble et al., 2020). Thus, the route of administration of MG was varied and the effects of 7-OH-MG, an active metabolite of MG at the MOR, were assessed.

Effects of Pretreated Compounds Alone. Prior to assessment of the above compounds in combination with antagonists at the opioid receptor and Aα_2R (naltrexone and yohimbine, respectively), the effects of the antinociception antagonists alone were studied. Neither naloxone nor yohimbine significantly change rates of responding (Figure S3; Table S9). There was a significant effect of yohimbine (and not naltrexone) on rectal temperature, i.e., 3.2 mg/kg yohimbine significantly decreased rectal temperature (Figure S3; Table S9). There was a significant effect of sex on rectal temperature for both naltrexone and yohimbine (Table S9). However, only the effects of 3.2 mg/kg yohimbine on rectal temperature were significant (Table S9).

Naltrexone (0.032 mg/kg) significantly shifted to the right the dose-effect function of methadone to produce rate-decreasing effects 5-fold (Figure 4; Table 2). Yohimbine (3.2 mg/kg) did not significantly modify the effects of methadone on rates of responding, antinociception, and changes in rectal temperature (Figure 4; Table S4). There was no significant effect of sex or interaction of sex with dose (Table S4).

U69,593 in Combination with Naltrexone or Yohimbine. Naltrexone (0.032 mg/kg) produced a small but statistically significant leftward shift in the dose-effect functions of U69,593 for rate-decreasing effects, and did not modify the antinociceptive or hypothermic of U69,593 (Figure 4; Tables 2 and S6). Naltrexone (1.0 mg/kg) significant antagonized the rate-decreasing, antinociceptive, and hypothermic effects of U69,593 (Figure 4; Tables 2, S6). Significant rightward shifts were obtained for the rate-decreasing and antinociceptive effects of U69,593 (5- and 3-fold, Table 3). At each dose of naltrexone, there was no significant effect of sex or interaction of sex with U69,593 dose (Table S6).

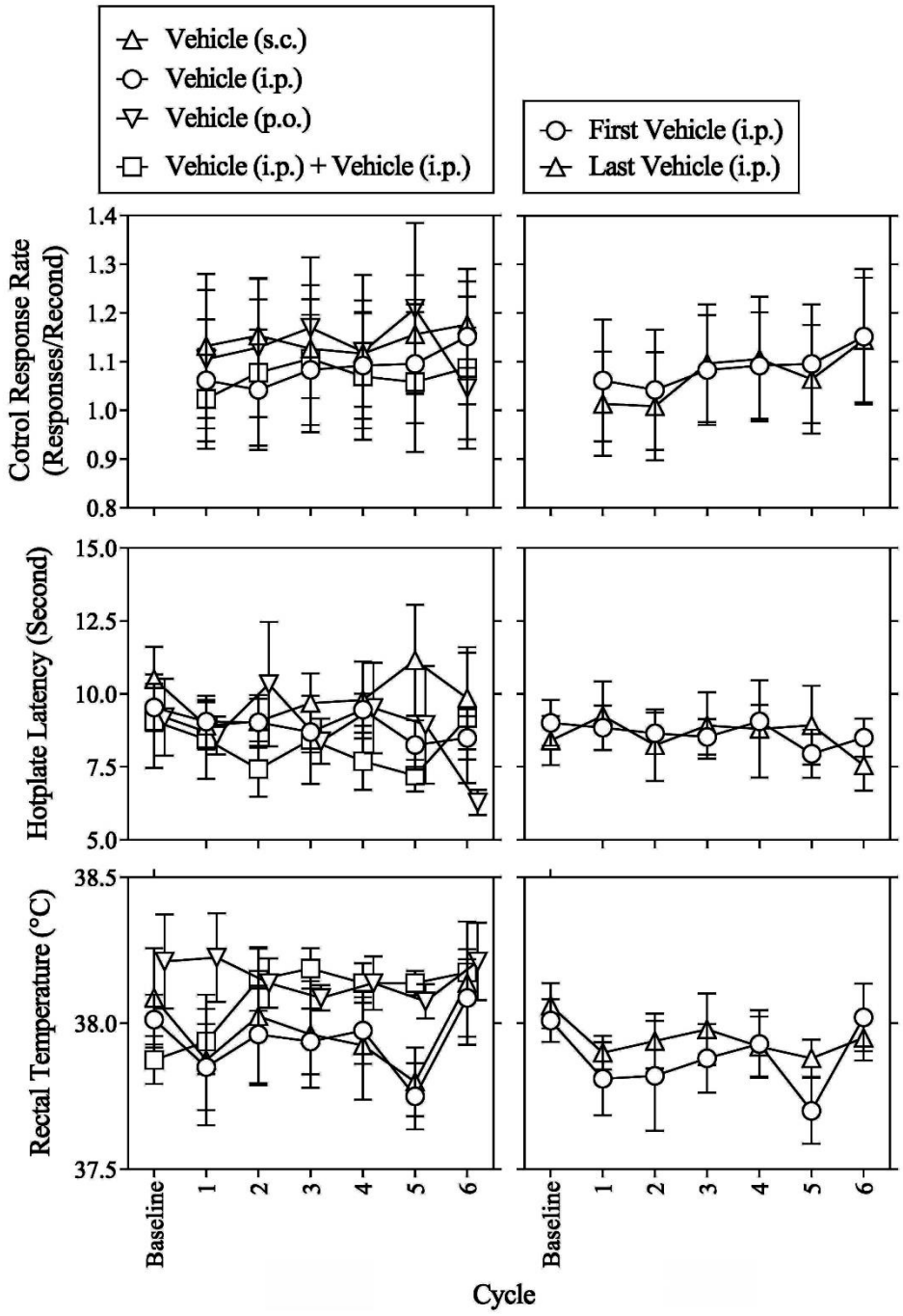
MG (i.p.) and 7-OH-MG in Combination with Naltrexone or Yohimbine For naltrexone, there was no significant effect of sex or interaction of sex with MG dose on rates of responding, antinociception, or changes in rectal temperature (Table S10). For yohimbine, there was no significant effect of sex or interaction of sex with MG dose on rates of responding or antinociception (Table S10). However, there was no significant effect of sex but a significant effect of interaction of sex with MG dose on rectal temperature (Table S10); nonetheless, a *post hoc* test indicated no significant difference in rectal temperature across sex (Table S10). Naltrexone (0.032 mg/kg) significantly shifted the dose-effect functions of 7-OH-MG 3-fold rightward for both rate-decreasing and antinociceptive effects (Figure 5; Tables 2, 3, and S9). In contrast, yohimbine (3.2 mg/kg) did not significantly modify the rate-decreasing or antinociceptive dose-effect functions of 7-OH-MG (Figure 5; Tables 2 and S9). For naltrexone, there was no significant effect of sex but a significant effect of interaction of sex with 7-OH-MG dose on rates of responding (Table S9). Nonetheless, a *post hoc* test indicated no significant difference in rates of responding across sex (Table S9). In addition, there was no significant effect of sex or interaction of sex with 7-OH-MG dose on antinociception or rectal temperature (Table S9). For yohimbine, there was no significant effect of sex or interaction of sex with 7-OH-MG dose on rates of responding, antinociception, or rectal temperature (Table S9).

For lofexidine in combination with MG, no significant difference in sex was found in rates of responding, antinociception or rectal temperature (Table S9). When clonidine was combined with MG, no significant difference in sex was found in antinociception (Table S9). In contrast to MG, 7-OH-MG did not significantly shift the dose-effect function of the hypothermic effects of lofexidine or clonidine (Figure 6; Tables 2 and S9).

Combinations of the Reference Agonists. When the morphine to lofexidine ratios were decreased, the dose-effect functions of rate-decreasing effects for morphine and lofexidine shifted to the left and right, respectively (Figure S4). When the morphine to lofexidine ratios were decreased, antinociception and the maximum decreases in rectal temperature were less (Figures S4 and S5). Similar changes were reproduced when the morphine to clonidine ratios were decreased (Figure S5).

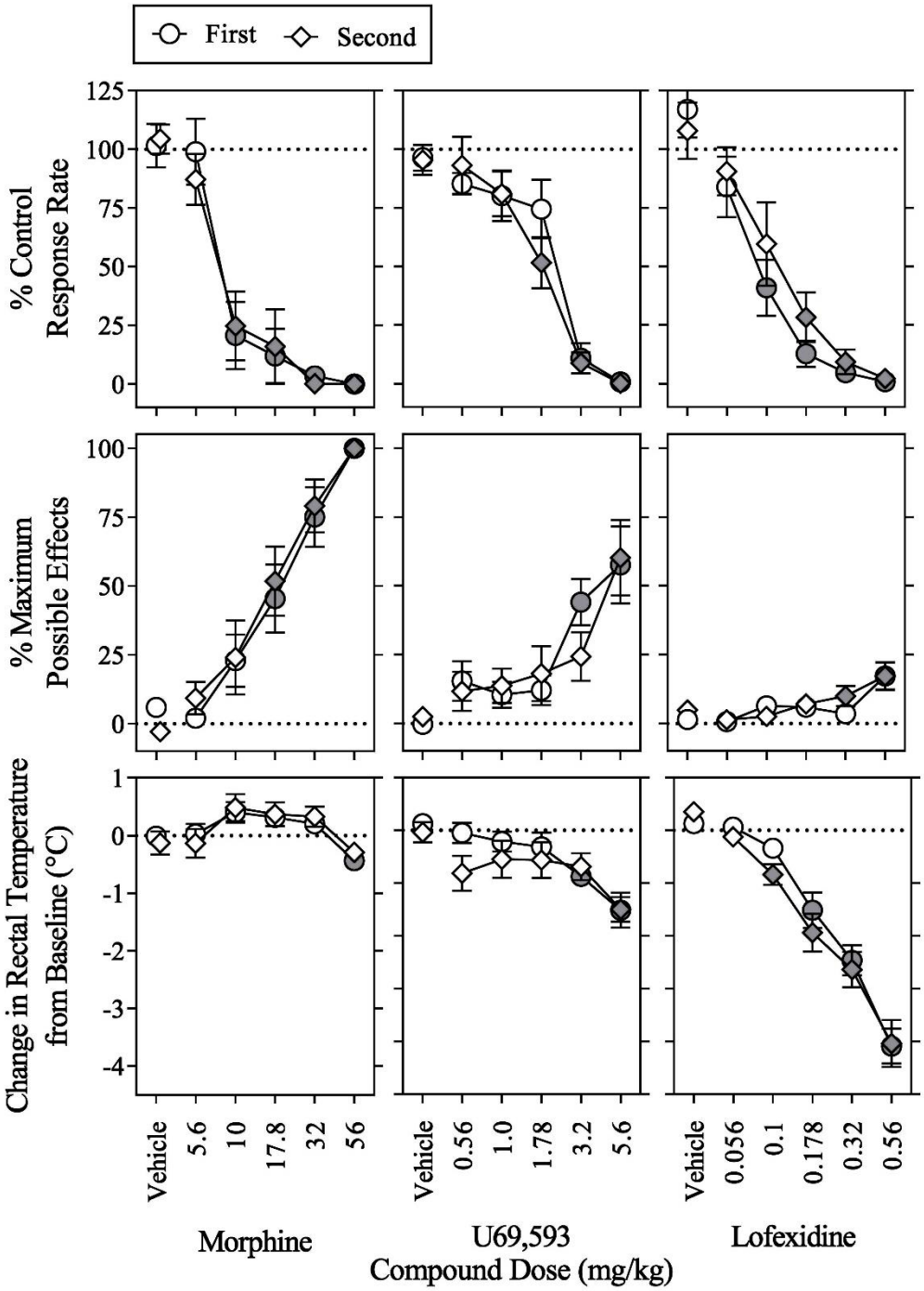
When the methadone to lofexidine ratios were decreased, antinociception and the maximum decreases in rectal temperature were less (Figure S6). Similar changes were reproduced when the methadone to clonidine ratios were decreased (Figure S7). No significant difference in sex was obtained when methadone was combined with lofexidine or clonidine (Table S10).

When the U69,593 to lofexidine ratios were decreased, the maximum antinociception and the maximum decreases in rectal temperature were less (Figures S8). Similar changes were reproduced when the U69,593 to clonidine ratios were decreased (Figure S9). No significant difference in sex was obtained when U69,593 was combined with lofexidine or clonidine (Table S10).



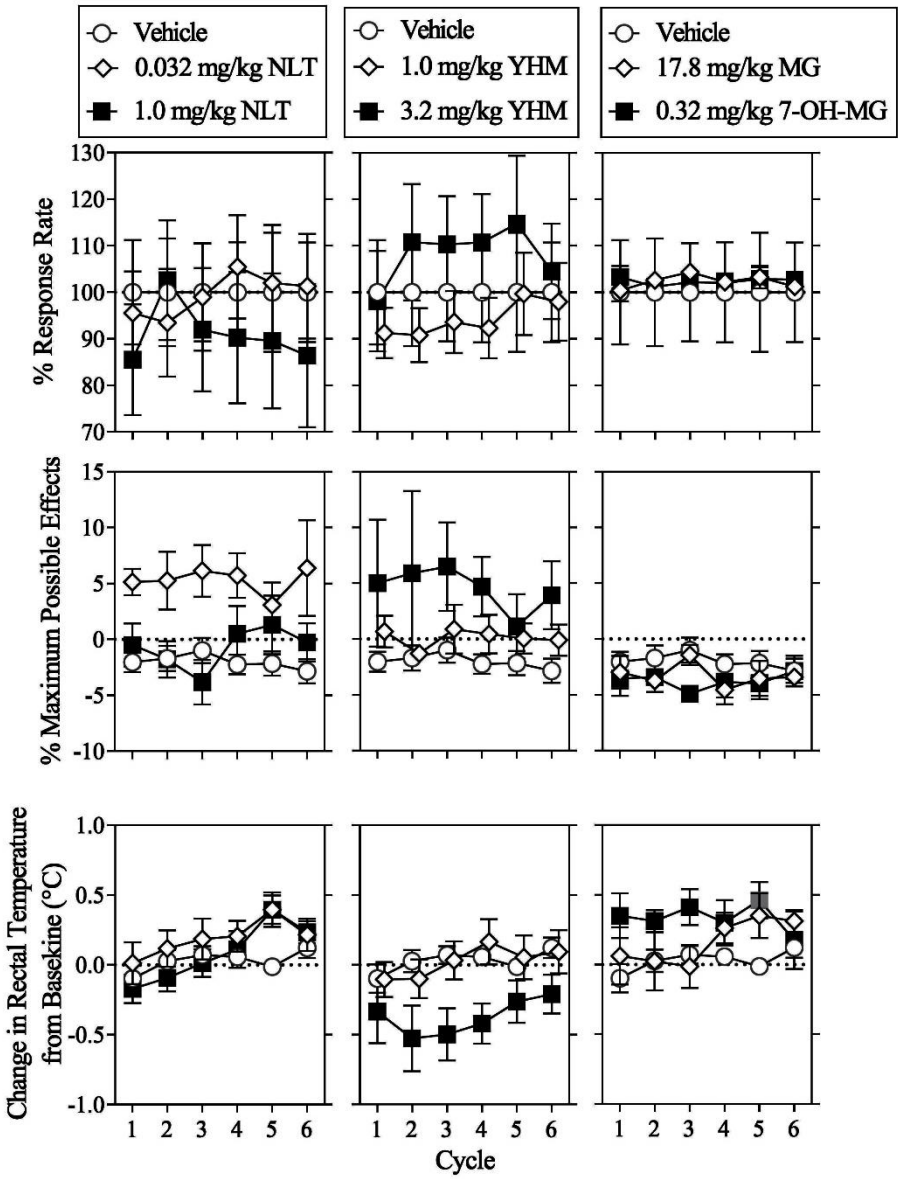
Supp. Figure 1. Effects of repeated injections of vehicle on rates of food-maintained behavior, hotplate response latency, and rectal temperature. Abscissae: Baseline and experimental cycle per session. Ordinates: *Top panels*, percentage of food-maintained lever responding expressed as a percentage of mean control values during inter-test sessions. *Middle panels*, hotplate response latency in second. *Bottom panels*, rectal temperature in °C.

Vehicle was administered through various routes of administration (i.p., s.c., and p.o. alone, or i.p. combined with i.p. at 15 min before each 5-minute period for data collection for food-maintained behavior. Each point represents the mean ± SEM (four rats/sex per group). *Left panels*, effects of first assessment only. *Right panels*, first and last assessments for the effects of repeated administration of i.p. vehicle. Note that no significant effects of routes of administration or reassessment were found.



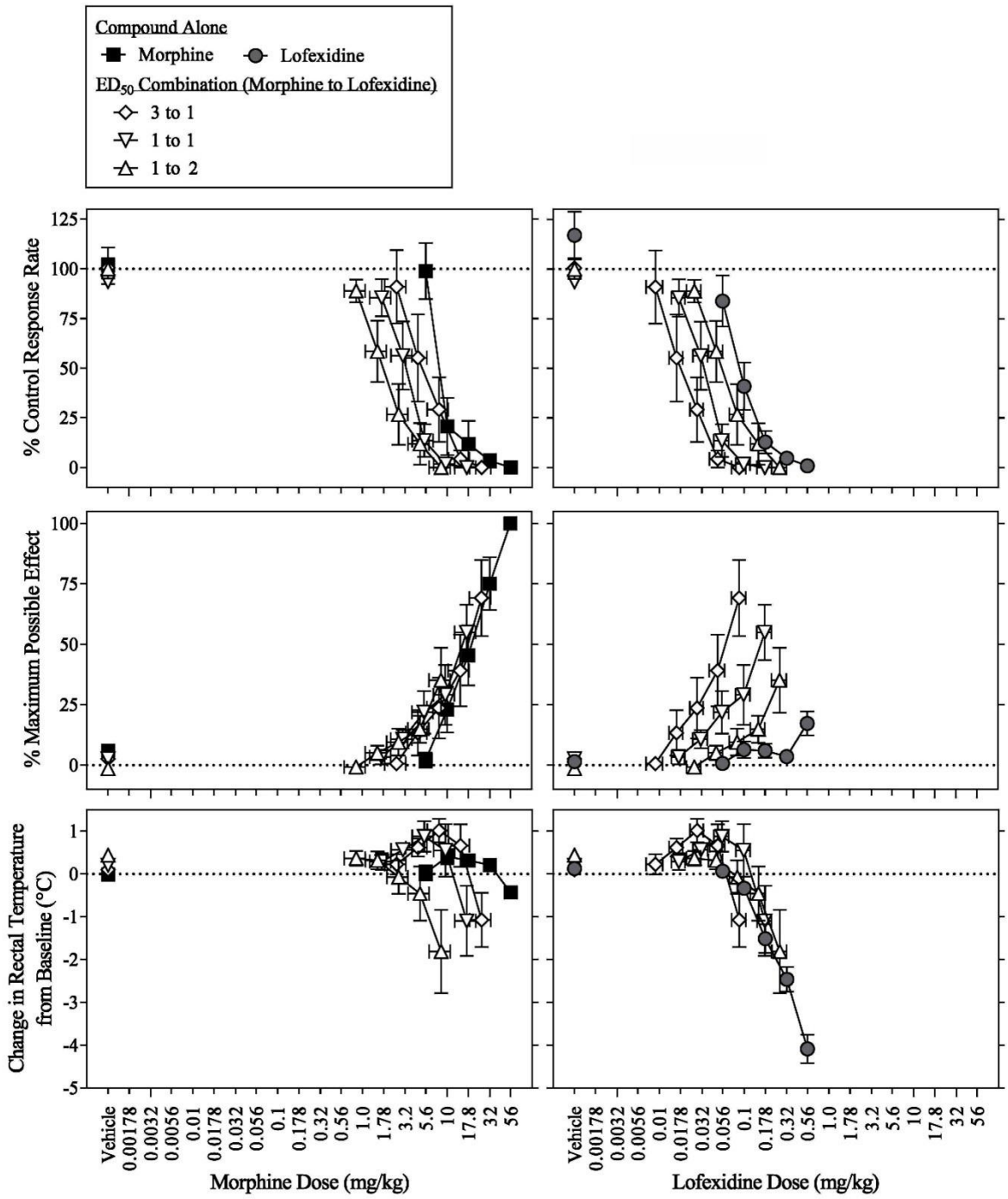
Supp. Figure S2. Stability of the effects of morphine, U69,593, and lofexidine on food-maintained behavior, antinociceptive maximum possible effects, and rectal temperature across two different days (prior to and following completion of all other test items). Abscissae: Vehicle and cumulative dose of compound. Circles and diamonds indicate first and second assessments, respectively. Ordinates: *Top panels*, percentage of food-

maintained responding expressed as a percentage of mean control values during inter-test sessions. *Middle panels*, percentage of maximum possible effects. *Bottom panels*, changes in rectal temperature in °C. Vehicle and each dose of morphine, U69,593, and lofexidine were administered i.p. at 15 min before each 5-minute period for data collection for food-maintained behavior. Each point represents the mean ± SEM (four rats per sex per group). *Left panels*, effects of morphine (vehicle and 5.6, 10, 17.8, 32, and 56 mg/kg). *Middle panels*, effects of U69,593 (vehicle and 0.56, 1.0, 1.78, 3.2, and 5.6 mg/kg). *Right panels*, effects of lofexidine (vehicle and 0.056, 0.1, 0.178, 0.32, and 0.56 mg/kg). Each gray symbol indicates a significant difference from vehicle per corresponding cycle as shown **Figure 3**. Note that no tolerance to the effects of any test compounds was found (**Tables S5-S7**).



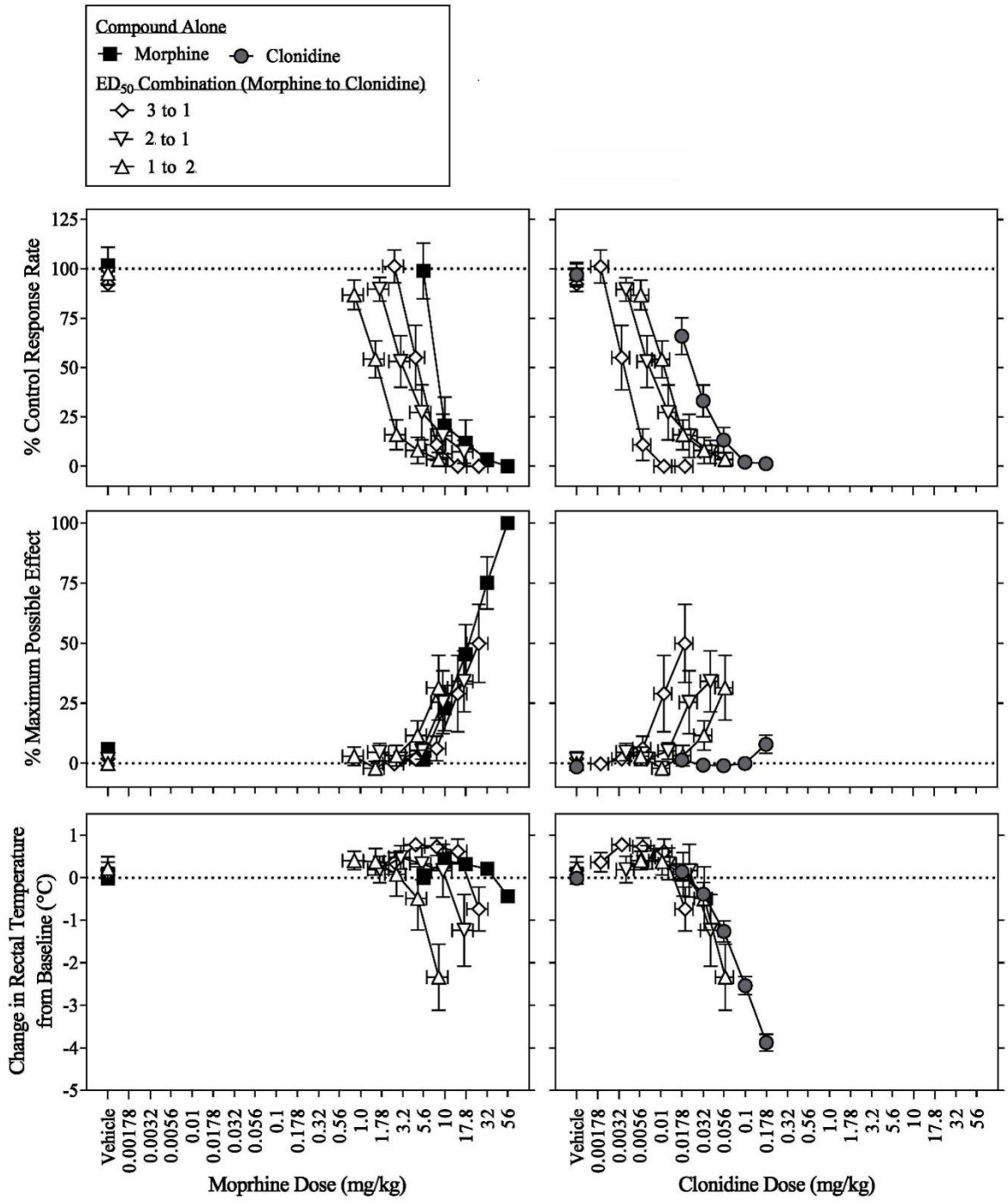
Supp. Figure 3. Effects of pretreatment with naltrexone (NLT), yohimbine (YHM), mitragynine (MG), or 7-hydroxymitragynine (7-OH-MG) on food-maintained behavior, antinociceptive maximum possible effects, and rectal temperature. Abscissae: experimental cycle. Ordinates: *Top panels*, percentage of food-maintained responding expressed as a percentage of mean control values during inter-test sessions. *Middle panels*, percentage of maximum possible effects. *Bottom panels*, changes in rectal temperature in °C. Each dose of test compounds and vehicle were administered i.p. once immediately before respective sessions. Each point represents the mean ± SEM (four rats per sex per group). *Left panels*, effects of vehicle (open circles) and

naltrexone [0.032 (open diamonds) and 1.0 mg/kg (filled squares)]. *Middle panels*, effects of vehicle (open circles, duplication from left panels) and yohimbine [1.0 (open diamonds) and 3.2 mg/kg (filled squares)]. *Right panels*, effects of vehicle (open circles, duplication from left panels), 17.8 mg/kg mitragynine (open diamonds), and 0.32 mg/kg 7-hydroxymitragynine (filled squares). Note that there was no significant effects of any test compounds except yohimbine relative to vehicle per cycle.



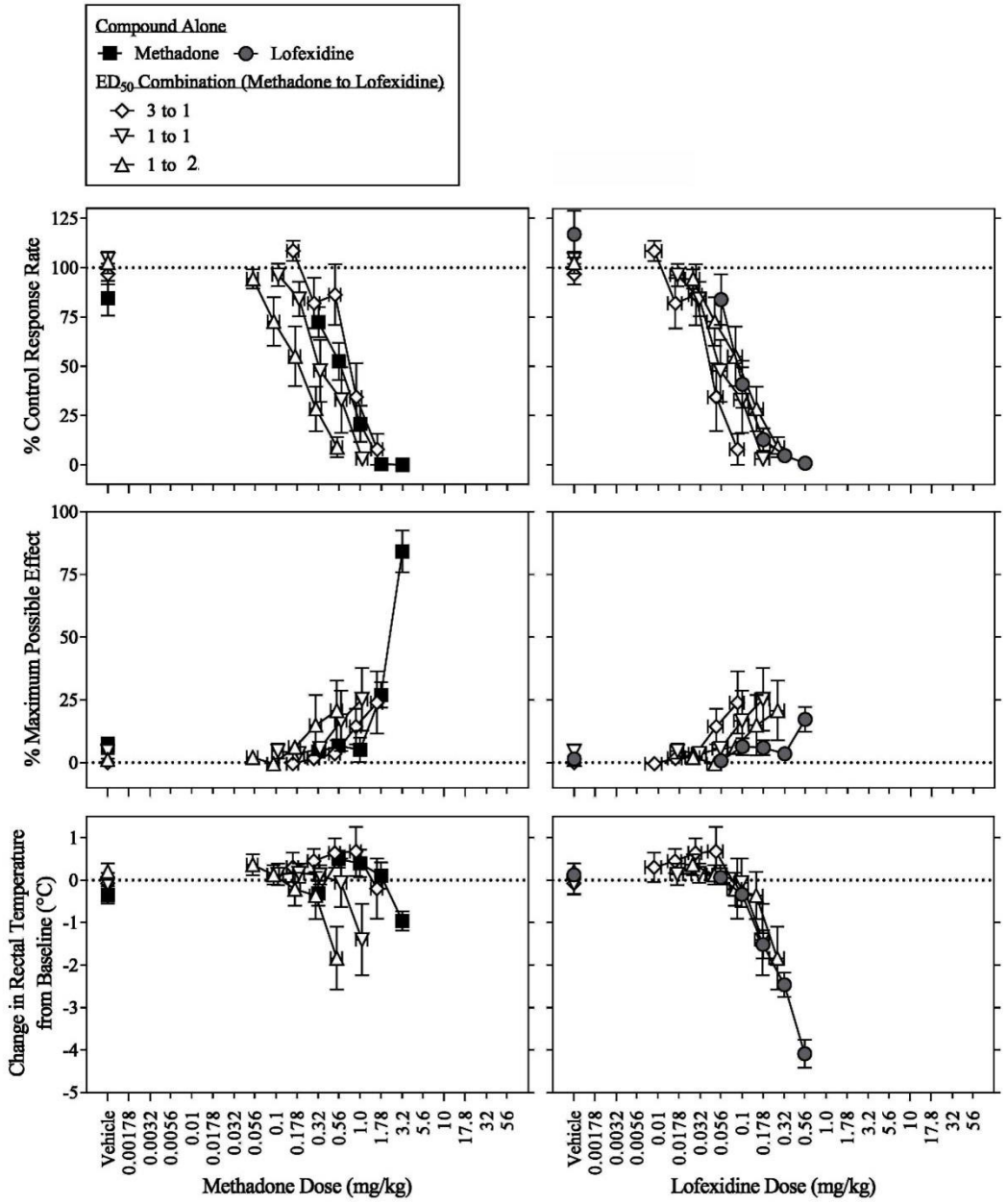
Supp. Figure 4. The rate-decreasing, antinociceptive, and hypothermic effects of combinations of morphine with lofexidine. Abscissae: Vehicle and cumulative dose of compound in mg/kg (i.p., log scale). *Left*, morphine dose; *right*, lofexidine dose. Ordinates: *Top row*, percentage of mean rates of responding after repeated administration of vehicle during inter-test sessions; *middle low*, percentage of maximum possible effects in the hot plate assay; *bottom row*, changes in rectal temperature from mean baselines. Each point

represents the mean ± SEM (N=4 per sex per data point). Vehicle, morphine, and lofexidine were administered i.p. immediately before each 5-minute period for data collection for food-maintained behavior. Each data of compounds alone was replotted from Figure 3. *Left panels*: The effects of morphine alone and in combination with lofexidine. Morphine dose alone (black squares) and in ED_{50} value ratios of morphine and lofexidine 3:1 (diamonds), 1:1 (downward triangles), and 1:2 (upward triangles). *Right panels*: The effects of lofexidine alone and in combination with morphine. Lofexidine dose alone (gray circles) and in the ED_{50} value ratios of morphine and lofexidine 3:1 (diamonds), 1:1 (downward triangles), and 1:2 (upward triangles). Each data of the combinations was replotted from the corresponding left panels. Note that the leftward shifts in the dose-effect functions of morphine and lofexidine for food-maintained behavior was obtained when morphine and lofexidine were combined.



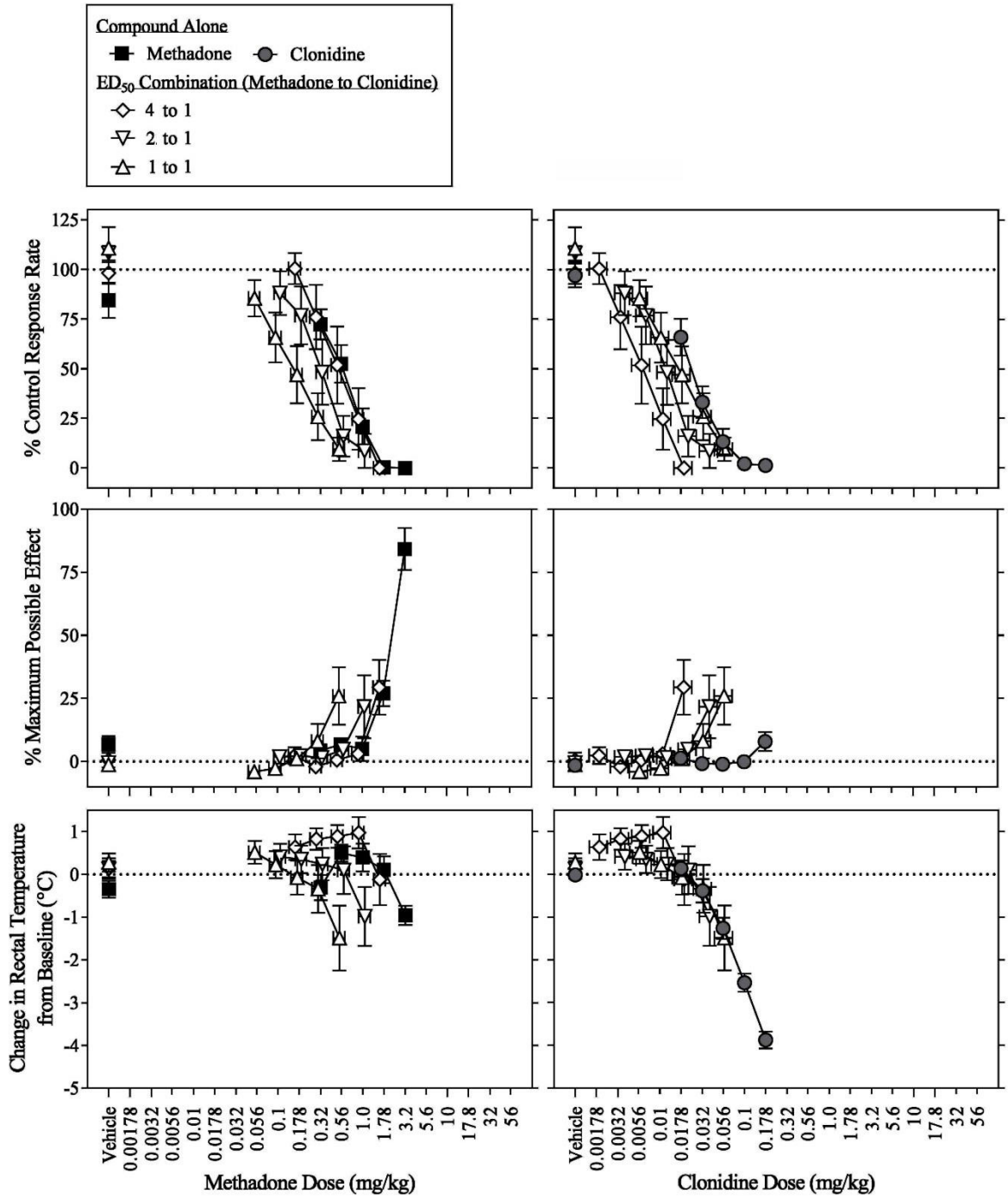
Supp. Figure 5. The rate-decreasing, antinociceptive, and hypothermic effects of combinations of morphine with clonidine. Abscissae: Vehicle and cumulative dose of compound in mg/kg (i.p., log scale). *Left*, morphine dose; *right*, clonidine dose. Ordinates: *Top row*, percentage of mean rates of responding after repeated vehicle administration during inter-test sessions; *middle low*, percentage of maximum possible effects in the hot plate

assay; *bottom row*, changes in rectal temperature from mean baselines. Each point represents the mean ± SEM (N=4 per sex per data point). Vehicle, morphine, and clonidine were administered i.p. immediately before each 5-minute period for data collection for food-maintained behavior. Each data of compounds alone was replotted from Figure 3. *Left panels*: The effects of morphine alone and in combination with clonidine. Morphine dose alone (black squares) and in ED_{50} value ratios of morphine and clonidine 3:1 (diamonds), 2:1 (downward triangles), and 1:2 (upward triangles). *Right panels*: The effects of clonidine alone and in combination with morphine. Clonidine dose alone (gray circles) and in the ED_{50} value ratios of morphine and clonidine 3:1 (diamonds), 2:1 (downward triangles), and 1:2 (upward triangles). Each data of the combinations was replotted from the corresponding left panels.



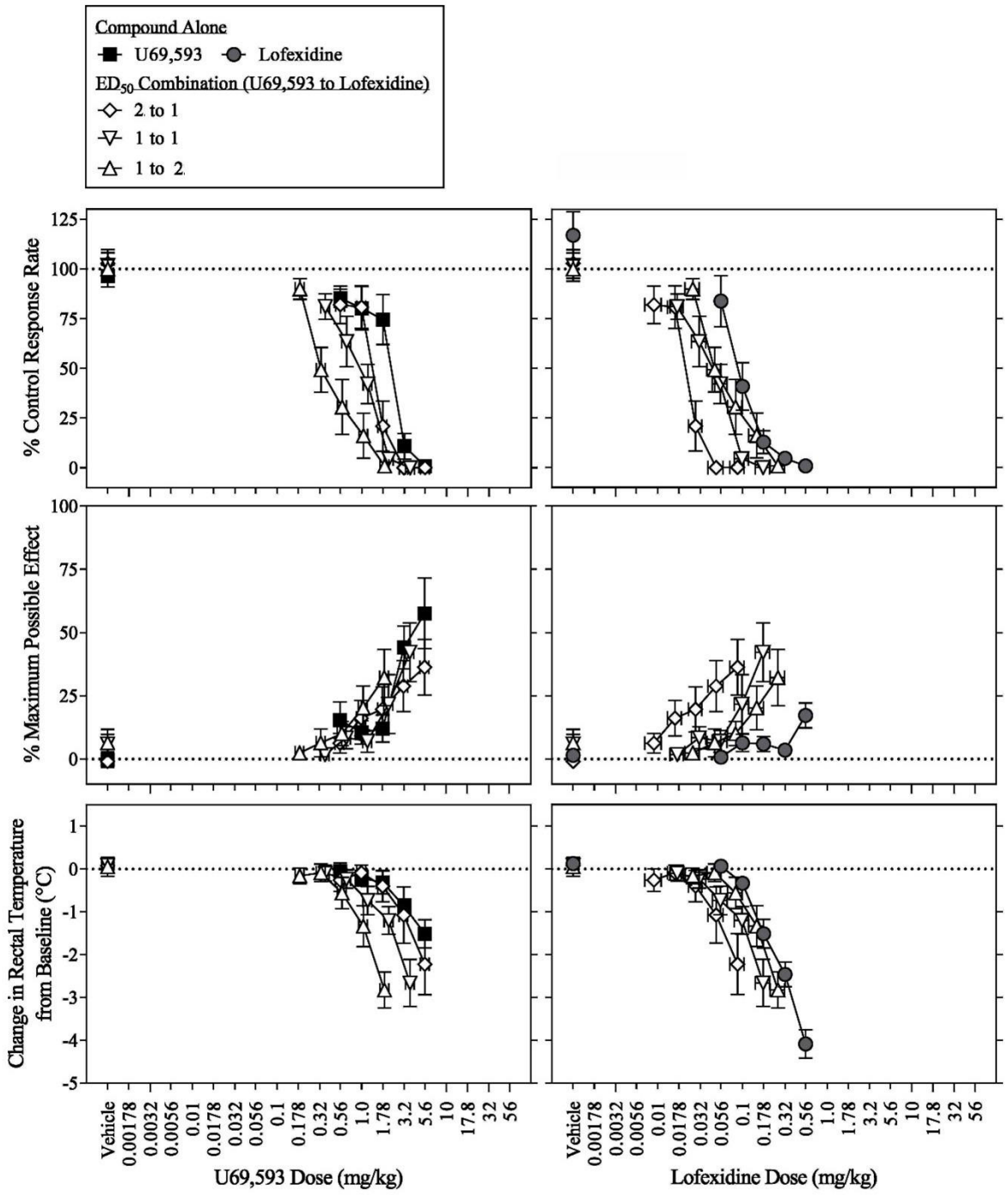
Supp. Figure 6. The rate-decreasing, antinociceptive, and hypothermic effects of combinations of methadone with lofexidine. Abscissae: Vehicle and cumulative dose of compound in mg/kg (i.p., log scale). *Left*, methadone dose; *right*, lofexidine dose. Ordinates: *Top row*, percentage of mean rates of responding after repeated administration of vehicle during inter-test sessions; *middle low*, percentage of maximum possible effects in the hot plate assay; *bottom row*, changes in rectal temperature from mean baselines. Each point represents the mean ± SEM (N=4 per sex per data point). Vehicle, methadone, and lofexidine were administered i.p. immediately before each 5-minute period for data collection for food-maintained behavior.

Each data of compounds alone was replotted from Figure 3. *Left panels*: The effects of methadone alone and in combination with lofexidine. Methadone dose alone (black squares) and in ED_{50} value ratios of methadone and lofexidine 3:1 (diamonds), 1:1 (downward triangles), and 1:2 (upward triangles). *Right panels*: The effects of lofexidine alone and in combination with methadone. Lofexidine dose alone (gray circles) and in the ED_{50} value ratios of methadone and lofexidine 3:1 (diamonds), 1:1 (downward triangles), and 1:2 (upward triangles). Each data of the combinations was replotted from the corresponding left panels.



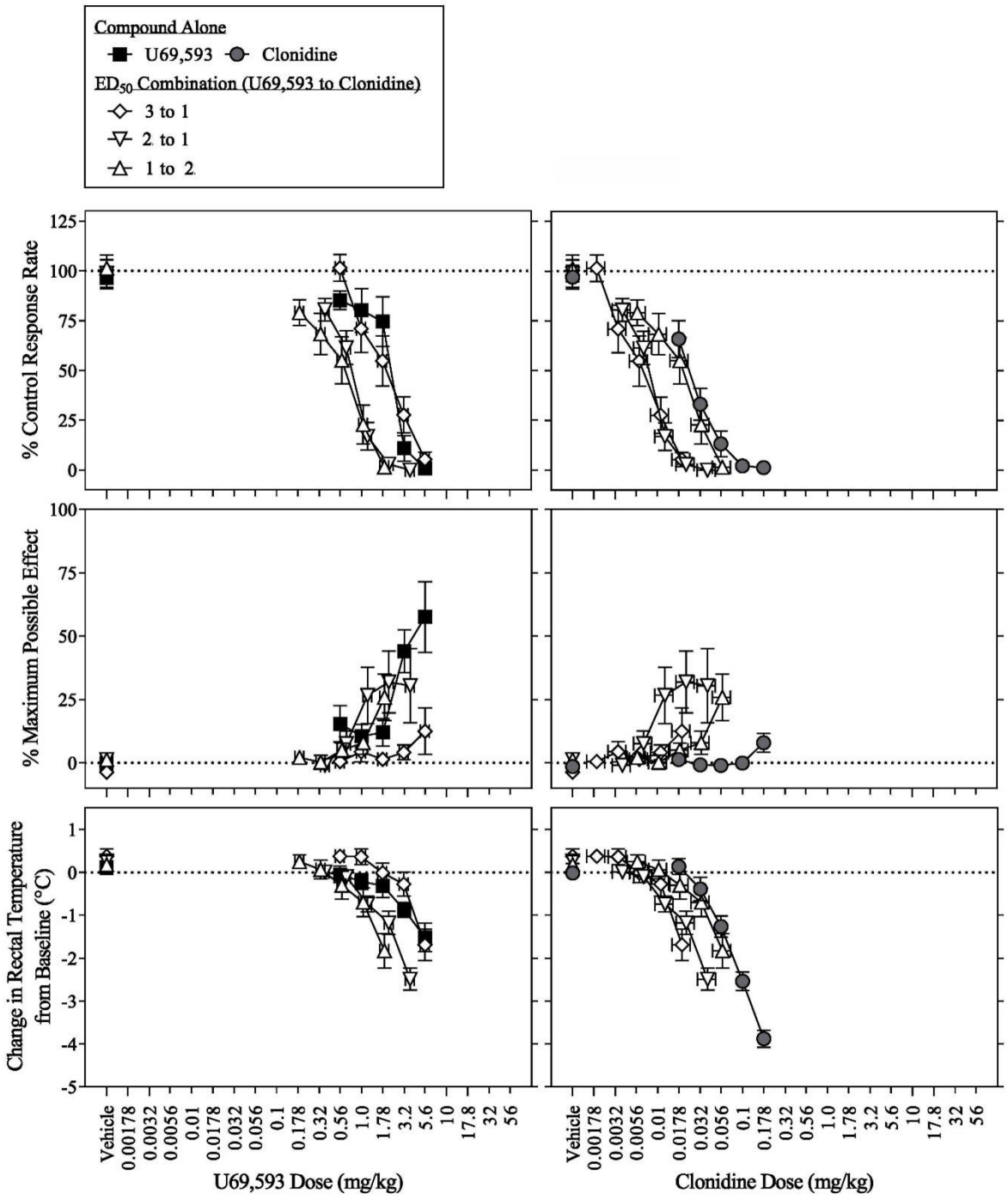
Supp. Figure 7. The rate-decreasing, antinociceptive, and hypothermic effects of combinations of methadone with clonidine. Abscissae: Vehicle and cumulative dose of compound in mg/kg (i.p., log scale). *Left*, methadone dose; *right*, clonidine dose. Ordinates: *Top row*, percentage of mean rates of responding after repeated administration of vehicle during inter-test sessions; *middle low*, percentage of maximum possible effects in the hot plate assay; *bottom row*, changes in rectal temperature from mean baselines. Each point

represents the mean ± SEM (N=4 per sex per data point). Vehicle, methadone, and clonidine were administered i.p. immediately before each 5-minute period for data collection for food-maintained behavior. Each data of compounds alone was replotted from Figure 3. *Left panels*: The effects of methadone alone and in combination with clonidine. Methadone dose alone (black squares) and in ED_{50} value ratios of methadone and clonidine 4:1 (diamonds), 2:1 (downward triangles), and 1:1 (upward triangles). *Right panels*: The effects of clonidine alone and in combination with methadone. Clonidine dose alone (gray circles) and in the ED_{50} value ratios of methadone and clonidine 4:1 (diamonds), 2:1 (downward triangles), and 1:1 (upward triangles). Each data of the combinations was replotted from the corresponding left panels.



Supp. Figure 8. The rate-decreasing, antinociceptive, and hypothermic effects of combinations of U69,593 with lofexidine. Abscissae: Vehicle and cumulative dose of compound in mg/kg (i.p., log scale). *Left*, U69,593 dose; *right*, lofexidine dose. Ordinates: *Top row*, percentage of mean rates of responding after repeated administration of vehicle during inter-test sessions; *middle low*, percentage of maximum possible effects in the

hot plate assay; *bottom row*, changes in rectal temperature from mean baselines. Each point represents the mean ± SEM (N=4 per sex per data point). Vehicle, U69,593, and lofexidine were administered i.p. at 15 minutes before each 5-minute period for data collection for food-maintained behavior. Each data of compounds alone was replotted from Figure 3. *Left panels*: The effects of U69,593 alone and in combination with lofexidine. U69,593 dose alone (black squares) and in ED_{50} value ratios of U69,593 and lofexidine 2:1 (diamonds), 1:1 (downward triangles), and 1:2 (upward triangles). *Right panels*: The effects of lofexidine alone and in combination with U69,593. Lofexidine dose alone (gray circles) and in the ED_{50} value ratios of U69,593 and lofexidine 2:1 (diamonds), 1:1 (downward triangles), and 1:2 (upward triangles). Each data of the combinations was replotted from the corresponding left panels.



Supp. Figure 9. The rate-decreasing, antinociceptive, and hypothermic effects of combinations of U69,593 with clonidine. Abscissae: Vehicle and cumulative dose of compound in mg/kg (i.p., log scale). *Left*, U69,593 dose; *right*, clonidine dose. Ordinates: *Top row*, percentage of mean rates of responding after repeated administration of vehicle during inter-test sessions; *middle low*, percentage of maximum possible effects in the

hot plate assay; *bottom row*, changes in rectal temperature from mean baselines. Each point represents the mean ± SEM (N=4 per sex per data point). Vehicle, U69,593, and clonidine were administered i.p. at 15 minutes before each 5-minute period for data collection for food-maintained behavior. Each data of compounds alone was replotted from Figure 3. *Left panels*: The effects of U69,593 alone and in combination with clonidine. U69,593 dose alone (black squares) and in ED_{50} value ratios of U69,593 and clonidine 3:1 (diamonds), 2:1 (downward triangles), and 1:2 (upward triangles). *Right panels*: The effects of clonidine alone and in combination with U69,593. clonidine dose alone (gray circles) and in the ED_{50} value ratios of U69,593 and clonidine 3:1 (diamonds), 2:1 (downward triangles), and 1:2 (upward triangles). Each data of the combinations was replotted from the corresponding left panel.

Supp. Table 1 Summary of scintillation counting conditions employed for assessing affinity at various binding sites in competition for the radioligands labeling human $A\alpha_2R$ and opioid receptor subtypes. Radioligand concentrations (nM) are shown as mean ± SEM. K_d (nM) and B_{max} (pmol/mg) values in parentheses are 95% confidence intervals.

Human Receptor	Cell	Radioligand	Radioligand concentration (nM), (Mean ± SEM)	Nonspecific Binding (10 μM)	Incubation Buffer	Incubation Time and Temperature	K_d (nM) (95%CI)	B_{max} (pmol/mg) (95%CI)
$A\alpha_{2A}R$	L-alpha-2A L-cells	[^{3}H]RX821002	1.80 ± 0.217	Lofexidine	TME buffer	60 minutes @ RT	1.79 (1.20—2.37)	1.72 (1.50—1.93)
$A\alpha_{2C}R$	L-alpha-2C L-cells	[^{3}H]RX821002	1.85 ± 0.176	Lofexidine	TME buffer	60 minutes @ RT	2.75 (1.56—3.95)	2.92 (2.32—3.52)
DOR	CHO cells	[^{3}H]DADLE	0.864 ± 0.035	SNC80	TME buffer	60 minutes @ RT	0.426 (0.272—0.580)	5.04 (4.54—5.53)
KOR	HEK-293 cells	[^{3}H]U69,593	1.60 ± 0.139	U69,593	TME buffer	60 minutes @ RT	1.44 (0.453—2.42)	4.98 (4.13—5.83)
MOR	CHO cells	[^{3}H]DAMGO	1.39 ± 0.217	Naltrexone	TME buffer	60 minutes @ RT	1.72 (0.652—2.79)	6.41 (5.07—7.74)

95%CI: 95% confidence interval.
Kd: Equilibrium dissociation constant.
Bmax: Maximum specific binding.
RT: room temperature.

Supp. Table 2. Effects of repeated injections of vehicle through various routes of administration (i.p., s.c., and p.o. alone, or i.p. combined with i.p.) across experimental cycles including baseline values (for hotplate latency and rectal temperature) on food-maintained responding, hotplate response latency, and rectal temperature, as shown in **Supp. Figure 1** (left panels). Each sample size is four rats per sex per group. Comparisons were made using a three-way repeated-measures mixed (between-subject sex and within-subject cycle and route) ANOVA. No *post hoc* test was conducted because no significant effects or interactions were found.

Factor	Food-Maintained Responding (Responses/Second)	Hotplate Response Latency (Second)	Rectal Temperature (°C)
Sex	$F_{1,6}$=1.90; P=0.218	$F_{1,6}$=0.016; P=0.903	$F_{1,6}$=1.64; P=0.247
Cycle	$F_{5,30}$=0.468; P=0.797	$F_{6,36}$=0.899; P=0.506	$F_{6,36}$=1.63; P=0.168
Route	$F_{3,18}$=0.455; P=0.717	$F_{3,18}$=0.798; P=0.511	$F_{3,18}$=1.32; P=0.298
Sex*Cycle	$F_{5,30}$=1.22; P=0.323	$F_{6,36}$=1.81; P=0.125	$F_{6,36}$=0.341; P=0.910
Sex*Route	$F_{3,18}$=1.77; P=0.189	$F_{3,18}$=0.779; P=0.521	$F_{3,18}$=1.53; P=0.242
Cycle*Route	$F_{15,90}$=0.837; P=0.634	$F_{18,108}$=1.11; P=0.349	$F_{18,108}$=1.34; P=0.180
Sex*Cycle*Route	$F_{15,90}$=0.956; P=0.507	$F_{18,108}$=0.956; P=0.514	$F_{18,108}$=0.892; P=0.590

Supp. Table 3. Stability assessment of effects of repeated administration of vehicle (i.p.) across two different days (prior to and following completion of all other test items) including baseline values (for hotplate latency and rectal temperature) on food-maintained responding, hotplate response latency, and rectal temperature, as shown in **Supp. Fig. 1** (right panels). Each sample size is four rats per sex per group. Comparisons were made using a three-way repeated-measures mixed (between-subject sex and within-subject cycle and day) ANOVA. No *post hoc* test was conducted because no significant effects or interactions were found per cycle.

Vehicle			
Factor	Food-Maintained Responding (Responses/Second)	Hotplate Response Latency (Second)	Rectal Temperature (°C)
Sex	$F_{1,6}$=1.42; P=0.279	$F_{1,6}$=0.397; P=0.552	$F_{1,6}$=0.450; P=0.527
Cycle	$F_{5,30}$=2.54; P=0.0597	$F_{6,36}$=0.686; P=0.662	$F_{6,36}$=1.59; P=0.178
Day	$F_{0.667,4.00}$=0.445; P=0.463	$F_{0.331,1.99}$=0.179; P=0.444	$F_{0.385,2.31}$=1.29; P=0.258
Sex*Cycle	$F_{5,30}$=2.97; P=0.0569	$F_{6,36}$=1.40; P=1.04	$F_{6,36}$=0.384; P=0.885
Sex*Day	$F_{1,6}$=0.103; P=0.759	$F_{1,6}$=0.0.000601; P=0.981	$F_{1,6}$=1.22; P=0.314
Cycle*Day	$F_{3.22,19.3}$=0.835; P=0.498	$F_{2.84,17.1}$=1.30; P=0.307	$F_{2.66,16.0}$=0.709; P=0.545
Sex*Cycle*Day	$F_{5,30}$=3.87; P=0.0579	$F_{6,36}$=2.07; P=0.0813	$F_{6,36}$=0.248; P=0.957

Supp. Table 4. Effects of the reference MOR agonists alone on food-maintained responding, antinociception, and changes in rectal temperature, as shown in **Figures 3** (upper panels), **4**, and **6**. Each sample size is four rats per sex per group. Comparisons relative to time-matching vehicle were made using a three-way repeated-measures mixed [between-subject sex and within-subject compound (compound or repeated vehicle) and compound dose] ANOVA followed by *post hoc* Bonferroni *t* tests with results shown only if there was a significant difference from the corresponding values per cycle unless noted. Statistically significant effects were shown in bold.

Morphine Alone (first assessment)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.338; P=0.582	$F_{1,6}$=0.668; P=0.445	$F_{1,6}$=0.205; P=0.669
Compound	$F_{0.466,2.79}$=25.4; **P=0.0201**	$F_{0.341,2.04}$=110; **P=0.0083**	$F_{0.502,0.487}$=0.259; P=0.487
Dose	$F_{5,30}$=28.5; **P<0.001**	$F_{5,30}$=27.7; **P<0.001**	$F_{5,30}$=4.85; **P=0.0023**
Sex*Compound	$F_{1,6}$=2.75; P=0.148	$F_{1,6}$=1.65; P=0.246	$F_{1,6}$=0.00647; P=0.939
Sex*Dose	$F_{5,30}$=1.74; P=0.156	$F_{5,30}$=1.19; P=0.338	$F_{5,30}$=0.963; P=0.456
Compound*Dose	$F_{1.94,11.7}$=28.5; **P<0.001**	$F_{1.68,10.1}$=30.1; **P<0.001**	$F_{2.74,16.4}$=7.73; **P=0.0023**
Sex*Compound*Dose	$F_{5,30}$=1.38; P=0.258	$F_{5,30}$=1.19; P=0.340	$F_{5,30}$=4.29; **P=0.0046**
Post Hoc	10 mg/kg (t=4.45, **P=0.004**) 17.8 mg/kg (t=5.57, **P<0.001**)	17.8 mg/kg (t=3.82. **P=0.0383**)	56 mg/kg (t=3.56, **P=0.0179)**

	32 mg/kg (t=7.28, **P<0.001**) 56 mg/kg (t=9.33, **P<0.001**)	32 mg/kg (t=7.06, **P=0.0011)** 56 mg/kg (t=95.1, **P<0.001)**	
Morphine Alone (reassessment)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.198; P=0.672	$F_{1,6}$=2.16; P=0.192	$F_{1,6}$=2.11; P=0.197
Compound	$F_{0.371,2.23}$=19.2; **P=0.0388**	$F_{0.5003,3.002}$=65.0; **P=0.0049**	$F_{0.592,3.55}$=0.161; P=0.579
Dose	$F_{5,30}$=32.4; **P<0.001**	$F_{5,30}$=35.6; **P<0.001**	$F_{5,30}$=6.31; **P<0.001**
Sex*Compound	$F_{1,6}$=2.59; P=0.159	$F_{1,6}$=2.94; P=0.137	$F_{1,6}$=1.33; P=0.294
Sex*Dose	$F_{5,30}$=1.27; P=0.302	$F_{5,30}$=2.05; P=0.1006	$F_{5,30}$=0.105; P=0.408
Compound*Dose	$F_{1.85,11.1}$=26.1; **P<0.001**	$F_{1.68,10.1}$=40.0; **P<0.001**	$F_{2.29,13.7}$=3.69; **P=0.0475**
Sex*Compound*Dose	$F_{5,30}$=1.04; P=0.414	$F_{5,30}$=2.12; P=0.910	$F_{5,30}$=0.714; P=0.618
Post Hoc	10 mg/kg (t=4.16, **P=0.007**) 17.8 mg/kg (t=4.39, **P=0.005**)	17.8 mg/kg (t=4.28. **P=0.0215**)	Not applicable

	32 mg/kg (t=7.80, **P<0.001**) 56 mg/kg (t=9.33, **P<0.001**)	32 mg/kg (t=8.46, **P=0.0003)** 56 mg/kg (t=95.1, **P<0.001)**	
0.032 mg/kg Naltrexone + Morphine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.787; P=0.409	$F_{1,6}$=0.233; P=0.647	$F_{1,6}$=0.428; P=0.537
Compound	$F_{0.439,2.63}$=33.4; **P=0.0163**	$F_{0.491,2.95}$=17.9; **P=0.0281**	$F_{0.544,3.27}$=5.21; P=0.0983
Dose	$F_{5,30}$=25.2; **P<0.001**	$F_{5,30}$=21.7; **P<0.001**	$F_{5,30}$=7.21; **P<0.001**
Sex*Compound	$F_{1,6}$=1.11; P=0.333	$F_{1,6}$=0.0377; P=0.853	$F_{1,6}$=4.33; P=0.0827
Sex*Dose	$F_{5,30}$=1.85; P=0.133	$F_{5,30}$=2.13; P=0.089	$F_{5,30}$=2.72; **P=0.0384**
Compound*Dose	$F_{2.83,17.0}$=19.7; **P<0.001**	$F_{2.73,16.4}$=21.6; **P<0.001**	$F_{2.74,16.4}$=7.73; **P=0.0023**
Sex*Compound*Dose	$F_{5,30}$=0.707; P=0.623	$F_{5,30}$=1.07; P=0.398	$F_{5,30}$=4.20; **P<0.001**
Post Hoc	32 mg/kg (t=4.53, **P=0.0030**) 56 mg/kg (t=8.88, **P<0.001**)	100 mg/kg (t=4.28, **P=0.0201)**	178 mg/kg (t=3.85, **P=0.0253)**

	100 mg/kg (t=8.14, **P<0.001**) 178 mg/kg (t=9.00, **P<0.001**)	178 mg/kg (t=7.71, **P<0.001)**	
1.0 mg/kg Naltrexone + Morphine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.0247; P=0.880	$F_{1,6}$=0.570; P=0.479	$F_{1,6}$=0.277; P=0.618
Compound	$F_{0.540,3.24}$=2.26; P=0.196	$F_{0.262,1.57}$=1.89; P=0.206	$F_{0.337,2.02}$=0.232; P=0.192
Dose	$F_{5,30}$=7.48; **P<0.001**	$F_{5,30}$=1.89; P=0.125	$F_{5,30}$=1.02; P=0.422
Sex*Compound	$F_{1,6}$=0.00289; P=0.959	$F_{1,6}$=0.00140; P=0.971	$F_{1,6}$=0.810; P=0.403
Sex*Dose	$F_{5,30}$=0.957; P=0.459	$F_{5,30}$=0.167; P=0.972	$F_{5,30}$=0.930; P=0.476
Compound*Dose	$F_{2.48,14.9}$=4.61; **P=0.0222**	$F_{1.39,8.31}$=1.80; P=0.222	$F_{1.65,9.92}$=1.51; P=0.263
Sex*Compound*Dose	$F_{5,30}$=0.588; P=0.709	$F_{5,30}$=0.132; P=0.984	$F_{5,30}$=0.998; P=0.436
Post Hoc	560 mg/kg (t=3.26, **P=0.0363**)	Not applicable	Not applicable
3.2 mg/kg Yohimbine + Morphine			

Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.337; P=0.583	$F_{1,6}$=0.0584; P=0.817	$F_{1,6}$=0.706; P=0.433
Compound	$F_{0.615,3.69}$=40.4; **P=0.0052**	$F_{0.527,3.46}$=6.99; **P=0.0697**	$F_{0.422,2.53}$=0.0787; P=0.572
Dose	$F_{5,30}$=14.1; **P<0.001**	$F_{5,30}$=13.3; **P<0.001**	$F_{5,30}$=8.19; **P<0.001**
Sex*Compound	$F_{1,6}$=0.0269; P=0.875	$F_{1,6}$=1.49; P=0.269	$F_{1,6}$=0.311; P=0.597
Sex*Dose	$F_{5,30}$=0.527; P=0.754	$F_{5,30}$=1.12; P=0.374	$F_{5,30}$=2.47; P=0.0567
Compound*Dose	$F_{2.44,14.6}$=21.5; **P<0.001**	$F_{2.55,15.3}$=13.2; **P<0.001**	$F_{2.10,12.6}$=2.70; P=0.104
Sex*Compound*Dose	$F_{5,30}$=0.603; P=0.698	$F_{5,30}$=0.385; P=0.855	$F_{5,30}$=0.273; P=0.924
Post Hoc	10 mg/kg (t=4.06, **P=0.0073**) 17.8 mg/kg (t=6.74, **P<0.001**) 32 mg/kg (t=7.29, **P<0.001**) 56 mg/kg (t=10.2, **P<0.001**)	56 mg/kg (t=5.48, **P=0.0041)**	Not applicable
17.8 mg/kg Mitragynine + Morphine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)

Sex	$F_{1,6}$=0.227; P=0.651	$F_{1,6}$=0.835; P=0.396	$F_{1,6}$=1.88; P=0.220
Compound	$F_{0.247,1.48}$=173; **P=0.0129**	$F_{0.439,2.64}$=127; **P=0.0031**	$F_{0.558,3.35}$=0.0264; P=0.735
Dose	$F_{5,30}$=29.3; **P<0.001**	$F_{5,30}$=54.5; **P<0.001**	$F_{5,30}$=1.27; P=0.304
Sex*Compound	$F_{1,6}$=0.0990; P=0.764	$F_{1,6}$=0.163; P=0.701	$F_{1,6}$=0.212; P=0.662
Sex*Dose	$F_{5,30}$=0.203; P=0.959	$F_{5,30}$=1.69; P=0.168	$F_{5,30}$=0.787; P=0.568
Compound*Dose	$F_{1.58,9.47}$=40.8; **P<0.001**	$F_{2.44,14.7}$=60.3; **P<0.001**	$F_{2.90,17.4}$=1.18; **P=0.346**
Sex*Compound*Dose	$F_{5,30}$=0.288; P=0.916	$F_{5,30}$=1.26; P=0.308	$F_{5,30}$=0.890; P=0.500
Post Hoc	5.6 mg/kg (t=4.35, **P=0.0174**) 10 mg/kg (t=4.12, **P=0.0255**) 17.8 mg/kg (t=15.4, **P<0.001**) 32 mg/kg (t=40.7, **P<0.001**) 56 mg/kg (t=63.2, **P<0.001**)	32 mg/kg (t=5.35, **P=0.0053)** 56 mg/kg (t=30.5, **P<0.001)**	Not applicable
0.32 mg/kg 7-Hydroxymitragynine + Morphine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)

Sex	$F_{1,6}$=5.77; P=0.0532	$F_{1,6}$=0.785; P=0.410	$F_{1,6}$=3.66; P=0.104
Compound	$F_{0.515,3.09}$=220; **P=0.0007**	$F_{0.270,1.62}$=62.9; **P=0.0229**	$F_{0.557,3.34}$=0.291; P=0.492
Dose	$F_{5,30}$=43.8; **P<0.001**	$F_{5,30}$=35.5; **P<0.001**	$F_{5,30}$=1.68; P=0.170
Sex*Compound	$F_{1,6}$=5.44; P=0.0585	$F_{1,6}$=0.0178; P=0.898	$F_{1,6}$=1.27; P=0.302
Sex*Dose	$F_{5,30}$=3.54; **P=0.0125**	$F_{5,30}$=0.322; P=0.896	$F_{5,30}$=0.977; P=0.448
Compound*Dose	$F_{2.42,14.5}$=35.3; **P<0.001**	$F_{1.54,9.26}$=39.5; **P<0.001**	$F_{2.48,14.9}$=2.22; P=0.136
Sex*Compound*Dose	$F_{5,30}$=2.45; P=0.0564	$F_{5,30}$=0.411; P=0.837	$F_{5,30}$=0.812; P=0.551
Post Hoc	17.8 mg/kg (t=4.33, **P=0.0184**) 32 mg/kg (t=8.19, **P<0.001**) 56 mg/kg (t=75.3, **P<0.001**)	32 mg/kg (t=4.04, **P=0.0284)** 56 mg/kg (t=38.5, **P<0.001)**	Not applicable
Methadone Alone			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=2.49; P=0.166	$F_{1,6}$=2.09; P=0.198	$F_{1,6}$=1.54; P=0.262
Compound	$F_{0.357,2.14}$=21.1; **P=0.0375**	$F_{0.492,2.95}$=135; **P=0.0018**	$F_{0.482,2.89}$=0.342; P=0.445

Dose	$F_{5,30}$=24.9; **P<0.001**	$F_{5,30}$=50.8; **P<0.001**	$F_{5,30}$=8.67; **P<0.001**
Sex*Compound	$F_{1,6}$=1.03; P=0.350	$F_{1,6}$=1.17; P=0.321	$F_{1,6}$=0.521; P=0.497
Sex*Dose	$F_{5,30}$=0.676; P=0.645	$F_{5,30}$=1.49; P=0.224	$F_{5,30}$=2.32; P=0.0681
Compound*Dose	$F_{1.95,11.7}$=27.0; **P<0.001**	$F_{2.39,14.4}$=50.1; **P<0.001**	$F_{3.01,18.1}$=11.0; **P=0.0002**
Sex*Compound*Dose	$F_{5,30}$=1.52; P=0.215	$F_{5,30}$=1.17; P=0.373	$F_{5,30}$=5.77; **P=0.0008**
Post Hoc	0.56 mg/kg (t=3.36, **P=0.0286**) 1.0 mg/kg (t=5.59, **P=0.0004**) 1.78 mg/kg (t=7.77, **P<0.001**) 3.2 mg/kg (t=9.33, **P<0.001**)	1.78 mg/kg (t=5.67, **P=0.0033**) 3.2 mg/kg (t=7.24, **P<0.001**)	3.2 mg/kg (t=4.54, **P=0.0098)**
0.032 mg/kg Naltrexone + Methadone			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=1.06; P=0.343	$F_{1,6}$=0.0298; P=0.869	$F_{1,6}$=1.33; P=0.293
Compound	$F_{0.446,2.68}$=34.0; **P=0.0155**	$F_{0.252,1.51}$=5.51; P=0.125	$F_{0.360,2.16}$=1.59; P=0.233
Dose	$F_{5,30}$=7.83; **P<0.001**	$F_{5,30}$=4.82; **P=0.0024**	$F_{5,30}$=1.29; P=0.293

Sex*Compound	$F_{1,6}$=0.820; P=0.400	$F_{1,6}$=0.0210; P=0.890	$F_{1,6}$=0.0695; P=0.801
Sex*Dose	$F_{5,30}$=0.361; P=0.871	$F_{5,30}$=0.291; P=0.914	$F_{5,30}$=0.0496; P=0.998
Compound*Dose	$F_{2.37,14.2}$=12.2; **P<0.001**	$F_{1.56,9.39}$=6.52; **P=0.0210**	$F_{1.59,9.35}$=0.827; P=0.439
Sex*Compound*Dose	$F_{5,30}$=1.25; P=0.313	$F_{5,30}$=0.388; P=0.853	$F_{5,30}$=0.0729; P=0.996
Post Hoc	5.6 mg/kg (t=4.56, **P=0.0027**) 10 mg/kg (t=9.00, **P<0.001**)	5.6 mg/kg (t=5.67, **P=0.0033**) 10 mg/kg (t=7.24, **P<0.001**)	Not applicable
3.2 mg/kg Yohimbine + Methadone			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=1.18; P=0.319	$F_{1,6}$=0.742; P=0.422	$F_{1,6}$=0.0513; P=0.828
Compound	$F_{0.582,3.49}$=8.98; P=0.0517	$F_{0.394,2.36}$=5.80; P=0.105	$F_{0.451,2.70}$=0.382; P=0.421
Dose	$F_{5,30}$=10.3; **P<0.001**	$F_{5,30}$=13.6; **P<0.001**	$F_{5,30}$=4.71; **P=0.0027**
Sex*Compound	$F_{1,6}$=0.00941; P=0.926	$F_{1,6}$=4.27; P=0.0844	$F_{1,6}$=3.06; P=0.131
Sex*Dose	$F_{5,30}$=0.329; P=0.892	$F_{5,30}$=0.977; P=0.448	$F_{5,30}$=5.86; **P=0.0007**
Compound*Dose	$F_{3.17,19.0}$=14.3; **P<0.001**	$F_{1.85,11.1}$=21.7; **P<0.001**	$F_{1.72,10.3}$=3.09; P=0.0938

Sex*Compound*Dose	$F_{5,30}$=0.818; P=0.546	$F_{5,30}$=1.09; P=0.385	$F_{5,30}$=0.188; P=0.965
Post Hoc	1.78 mg/kg (t=6.25, **P<0.001**) 3.2 mg/kg (t=10.2, **P<0.001**)	3.2 mg/kg (t=6.48, **P=0.0011**)	Not applicable
17.8 mg/kg Mitragynine + Methadone			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=5.32; P=0.0605	$F_{1,6}$=0.109; P=0.753	$F_{1,6}$=4.22; P=0.0856
Compound	$F_{0.512,3.07}$=313; **P<0.001**	$F_{0.232,1.39}$=31.9; **P=0.0459**	$F_{0.539,3.23}$=1.79; P=0.228
Dose	$F_{5,30}$=54.6; **P<0.001**	$F_{5,30}$=29.7; **P<0.001**	$F_{5,30}$=0.631; P=0.678
Sex*Compound	$F_{1,6}$=6.45; **P=0.0441**	$F_{1,6}$=0.252; P=0.634	$F_{1,6}$=0.708; P=0.433
Sex*Dose	$F_{5,30}$=2.92; **P=0.0289**	$F_{5,30}$=0.167; P=0.973	$F_{5,30}$=3.93; **P=0.0074**
Compound*Dose	$F_{2.24,13.4}$=51.0; **P<0.001**	$F_{1.21,7.23}$=28.4; **P=0.008**	$F_{1.87,11.2}$=3.69; P=0.0612
Sex*Compound*Dose	$F_{5,30}$=2.99; **P=0.0264**	$F_{5,30}$=0.314; P=0.900	$F_{5,30}$=6.56; **P<0.001**
Post Hoc	1.78 mg/kg (t=28.2, **P<0.001**)	56 mg/kg (t=19.5, **P<0.001)**	Not applicable

	3.2 mg/kg (t=63.2, **P<0.001**)		
0.32 mg/kg 7-Hydroxymitragynine + Methadone			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=3.82; P=0.0984	$F_{1,6}$=3.42; P=0.114	$F_{1,6}$=2.40; P=0.172
Compound	$F_{0.432,2.59}$=49.7; **P=0.0105**	$F_{0.336,2.02}$=426; **P=0.0023**	$F_{0.379,2.27}$=3.42; P=0.153
Dose	$F_{5,30}$=21.3; **P<0.001**	$F_{5,30}$=248; **P<0.001**	$F_{5,30}$=1.70; P=0.166
Sex*Compound	$F_{1,6}$=1.53; P=0.262	$F_{1,6}$=0.807; P=0.404	$F_{1,6}$=0.207; P=0.665
Sex*Dose	$F_{5,30}$=1.94; P=0.118	$F_{5,30}$=1.61; P=0.188	$F_{5,30}$=5.61; **P<0.001**
Compound*Dose	$F_{2.32,13.9}$=23.2; **P<0.001**	$F_{2.02,12.1}$=216; **P<0.001**	$F_{2.06,12.4}$=0.723; P=0.509
Sex*Compound*Dose	$F_{5,30}$=1.75; P=0.155	$F_{5,30}$=1.51; P=0.217	$F_{5,30}$=3.08; **P=0.0233**
Post Hoc	17.8 mg/kg (t=4.23, **P=0.0213**) 32 mg/kg (t=4.56, **P=0.0139**) 56 mg/kg (t=75.3, **P<0.001**)	32 mg/kg (t=10.1, **P<0.001**) 56 mg/kg (t=74.1, **P<0.001)**	Vehicle (t=4.69, **P=0.0025)**

Supp. Table 5. Stability assessment of effects of morphine, U69,593, and lofexidine across two different days (prior to and following completion of all other test items) on food-maintained responding, hotplate response latency, and rectal temperature, as shown in **Supp. Fig. 2.** Each sample size is four rats per sex per group. Comparisons were made using a three-way repeated-measures mixed (between-subject sex and within-subject dose and day) ANOVA. No *post hoc* test was conducted because no significant effects or interactions were found per dose.

Morphine			
Factor	Food-Maintained Responding (Responses/Second)	Hotplate Response Latency (Second)	Rectal Temperature (°C)
Sex	$F_{1,6}$=4.06; P=0.0905	$F_{1,6}$=2.30; P=0.180	$F_{1,6}$=0.610; P=0.465
Day	$F_{0.364,2.19}$=0.00491; P=0.720	$F_{0.363,2.18}$=0.159; P=0.473	$F_{0.620,3.72}$=0.0191; P=0.786
Dose	$F_{5,30}$=50.8; **P<0.001**	$F_{5,30}$=49.9; **P<0.001**	$F_{5,30}$=10.3; **P<0.001**
Sex*Day	$F_{1,6}$=0.0645; P=0.808	$F_{1,6}$=1.06; P=0.343	$F_{1,6}$=2.15; P=0.193
Sex*Dose	$F_{5,30}$=1.87; P=0.129	$F_{5,30}$=2.35; P=0.0649	$F_{5,30}$=1.71; P=0.162
Dose*Day	$F_{1.65,9.90}$=0.326; P=0.689	$F_{1.39,8.32}$=0.531; P=0.544	$F_{2.18,13.1}$=0.468; P=0.652
Sex*Day*Dose	$F_{5,30}$=0.708; P=0.622	$F_{5,30}$=1.66; P=0.176	$F_{5,30}$=0.643; P=0.669
U69,593			
Factor	Food-Maintained Responding (Responses/Second)	Hotplate Response Latency (Second)	Rectal Temperature (°C)
Sex	$F_{1,6}$=0.151; P=0.711	$F_{1,6}$=5.19; P=0.063	$F_{1,6}$=13.3; **P=0.0107**
Day	$F_{0.344,2.06}$=0.226; P=0.430	$F_{0.435,2.61}$=0.249; P=0.464	$F_{0.515,3.09}$=3.12; P=0.156
Dose	$F_{5,30}$=52.4; **P<0.001**	$F_{5,30}$=13.9; **P<0.001**	$F_{5,30}$=26.2; **P<0.001**
Sex*Day	$F_{1,6}$=0.106; P=0.756	$F_{1,6}$=0.471; P=0.518	$F_{1,6}$=3.48; P=0.111

Sex*Dose	$F_{5,30}$=0.844; P=0.530	$F_{5,30}$=3.27; **P=0.0179**	$F_{5,30}$=2.26; P=0.0736
Dose*Day	$F_{2.55,15.3}$=1.57; P=0.239	$F_{2.65,15.9}$=1.60; P=0.231	$F_{1.8711.2}$=2.53; P=0.126
Sex*Day*Dose	$F_{5,30}$=0.0389; P=0.999	$F_{5,30}$=1.04; P=0.415	$F_{5,30}$=1.15; P=0.357
Lofexidine			
Factor	Food-Maintained Responding (Responses/Second)	Hotplate Response Latency (Second)	Rectal Temperature (°C)
Sex	$F_{1,6}$=2.76; P=0.148	$F_{1,6}$=2.05; P=0.202	$F_{1,6}$=1.03; P=0.349
Day	$F_{0.390,2.34}$=0.950; P=0.294	$F_{0.356,2.14}$=0.658; P=0.327	$F_{0.394,2.37}$=1.97; P=0.211
Dose	$F_{5,30}$=30.1; **P<0.001**	$F_{5,30}$=9.19; **P<0.001**	$F_{5,30}$=89.2; **P<0.001**
Sex*Day	$F_{1,6}$=0.483; P=0.513	$F_{1,6}$=1.22; P=0.312	$F_{1,6}$=16.3; **P=0.0068**
Sex*Dose	$F_{5,30}$=1.26; P=0.305	$F_{5,30}$=1.85; P=0.134	$F_{5,30}$=1.10; P=0.381
Dose*Day	$F_{1.86,11.2}$=1.31; P=0.307	$F_{2.31,13.9}$=0.973; P=0.414	$F_{2.17,13.0}$=1.24; P=0.326
Sex*Day*Dose	$F_{5,30}$=0.126; P=0.985	$F_{5,30}$=0.129; P=0.985	$F_{5,30}$=1.16; P=0.354

Supp. Table 6 Effects of the reference KOR agonist U69,593 alone on food-maintained responding, antinociception, and changes in rectal temperature, as shown in **Figures 3** (upper panels), **4**, **6**, and **Supp. Fig. 2**. Each sample size is four rats per sex per group. Comparisons relative to time-matching vehicle were made using a three-way repeated-measures mixed [between-subject sex and within-subject compound (compound or repeated vehicle) and compound dose] ANOVA followed by *post hoc* Bonferroni *t* tests with results shown only if there was a significant difference from the corresponding values per cycle unless noted. Statistically significant effects were shown in bold.

U69,593 Alone (first assessment)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=2.44; P=0.169	$F_{1,6}$=6.77; **P=0.0405**	$F_{1,6}$=7.61; **P=0.0329**
Compound	$F_{0.490,2.94}$=10.6; P=0.0514	$F_{0.588,3.53}$=41.9; **P=0.0056**	$F_{0.439,2.64}$=14.6; **P=0.0414**
Dose	$F_{5,30}$=30.4; **P<0.001**	$F_{5,30}$=14.9; **P<0.001**	$F_{5,30}$=13.9; **P<0.001**
Sex*Compound	$F_{1,6}$=0.702; P=0.434	$F_{1,6}$=4.82; P=0.0706	$F_{1,6}$=5.55; P=0.0565
Sex*Dose	$F_{5,30}$=0.691; P=0.634	$F_{5,30}$=3.16; **P=0.0209**	$F_{5,30}$=0.770; P=0.579
Compound*Dose	$F_{2.64,15.9}$=32.8; **P<0.001**	$F_{2.68,16.1}$=13.0; **P<0.001**	$F_{1.95,11.7}$=16.5; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.701; P=0.627	$F_{5,30}$=2.45; P=0.0561	$F_{5,30}$=3.17; **P=0.0205**
Post Hoc 3.2 mg/kg (t=6.22, **P<0.001**) 5.6 mg/kg (t=9.26, **P<0.001**)	3.2 mg/kg (t=5.40, **P=0.0054)** 5.6 mg/kg (t=4.31, **P=0.0206)** *Sex* 5.6 mg/kg (t=4.13, **P<0.001)** *Female*	3.2 mg/kg (t=4.74, **P=0.0089)** 5.6 mg/kg (t=4.84, **P=0.0086)** *Sex* 3.2 mg/kg (t=2.21, **P=0.037)** 5.6 mg/kg (t=3.25, **P=0.003)** *Female*	

		3.2 mg/kg (t=4.71, **P<0.001)** 5.6 mg/kg (t=7.11, **P<0.001)**	1.78 mg/kg (t=2.98, **P=0.029)** 3.2 mg/kg (t=4.71, **P<0.001)** 5.6 mg/kg (t=7.44, **P<0.001)** *Male* 5.6 mg/kg (t=3.39, **P=0.010)**
U69,593 Alone (reassessment)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=1.58; P=0.256	$F_{1,6}$=4.10; P=0.0894	$F_{1,6}$=15.0; **P=0.0083**
Compound	$F_{0.501,23.00}$=11.9; **P=0.0434**	$F_{0.509,3.05}$=30.6; **P=0.0132**	$F_{0.488,2.93}$=15.9; **P=0.0326**
Dose	$F_{5,30}$=29.1; **P<0.001**	$F_{5,30}$=7.54; **P<0.001**	$F_{5,30}$=8.46; **P<0.001**
Sex*Compound	$F_{1,6}$=0.975; P=0.362	$F_{1,6}$=2.33; P=0.178	$F_{1,6}$=8.96; P=0.0242
Sex*Dose	$F_{5,30}$=0.477; P=0.791	$F_{5,30}$=2.58; **P=0.0469**	$F_{5,30}$=0.782; P=0.571
Compound*Dose	$F_{2.62,15.7}$=36.2; **P<0.001**	$F_{2.45,14.7}$=7.32; **P=0.0044**	$F_{2.47,14.8}$=13.4; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.454; P=0.807	$F_{5,30}$=2.25; P=0.0753	$F_{5,30}$=2.58; **P=0.0471**
Post Hoc	1.78 mg/kg (t=3.15, **P=0.0429**) 3.2 mg/kg (t=6.72, **P<0.001**)	5.6 mg/kg (t=4.57, **P=0.0149)**	5.6 mg/kg (t=6.52, **P<0.001)** *Sex* 0.56 mg/kg (t=3.17, **P=0.006)**

	5.6 mg/kg (t=9.31, **P<0.001**)		1.0 mg/kg (t=3.48, **P=0.003)** 1.78 mg/kg (t=3.79, **P=0.002)** 3.2 mg/kg (t=2.80, **P=0.014)** *Female* 0.56 mg/kg (t=3.89, **P=0.003)** 1.0 mg/kg (t=3.17, **P=0.018)** 1.78 mg/kg (t=3.44, **P=0.009)** 3.2 mg/kg (t=3.17, **P=0.018)** 5.6 mg/kg (t=5.52, **P<0.001)** *Male* 5.6 mg/kg (t=5.06, **P<0.001)**
0.032 mg/kg Naltrexone + U69,593			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=5.14; P=0.0639	$F_{1,6}$=0.256; P=0.631	$F_{1,6}$=0.271; P=0.622
Compound	$F_{0.403,2.42}$=18.1; **P=0.0370**	$F_{0.495,2.97}$=19.3; **P=0.0253**	$F_{0.338,2.03}$=13.7; P=0.0580

Dose	$F_{5,30}$=8.91; **P<0.001**	$F_{5,30}$=14.2; **P<0.001**	$F_{5,30}$=7.89; **P<0.001**
Sex*Compound	$F_{1,6}$=0.117; P=0.744	$F_{1,6}$=0.0392; P=0.850	$F_{1,6}$=2.34; P=0.177
Sex*Dose	$F_{5,30}$=0.110; P=0.989	$F_{5,30}$=0.316; P=0.899	$F_{5,30}$=0.780; P=0.572
Compound*Dose	$F_{1.72,10.3}$=21.0; **P<0.001**	$F_{2.10,12.6}$=17.9; **P<0.001**	$F_{1.56,9.33}$=15.2; **P=0.0017**
Sex*Compound*Dose	$F_{5,30}$=1.20; P=0.334	$F_{5,30}$=1.32; P=0.283	$F_{1.56,9.33}$=15.2; **P=0.0017**
Post Hoc	1.0 mg/kg (t=4.19, **P=0.0058**) 1.78 mg/kg (t=6.23, **P<0.001**) 3.2 mg/kg (t=7.56, **P<0.001**) 5.6 mg/kg (t=8.98, **P<0.001**)	3.2 mg/kg (t=6.11, **P=0.0024)** 5.6 mg/kg (t=5.69, **P=0.0021)**	1.78 mg/kg (t=3.93, **P=0.0222**) 3.2 mg/kg (t=3.55, **P=0.0484**) 5.6 mg/kg (t=3.93, **P=0.0381**)
1.0 mg/kg Naltrexone + U69,593			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.156; P=0.706	$F_{1,6}$=0.647; P=0.452	$F_{1,6}$=1.58; P=0.255
Compound	$F_{0.567,3.16}$=1.25; P=0.279	$F_{0.369,2.21}$=29.6; **P=0.0258**	$F_{0.339,2.04}$=4.72; P=0.127
Dose	$F_{5,30}$=9.11; **P<0.001**	$F_{5,30}$=26.2; **P<0.001**	$F_{5,30}$=4.64; **P=0.0030**
Sex*Compound	$F_{1,6}$=0.0223; P=0.886	$F_{1,6}$=2.84; P=0.143	$F_{1,6}$=1.84; P=0.224
Sex*Dose	$F_{5,30}$=0.734; P=0.554	$F_{5,30}$=2.00; P=0.107	$F_{5,30}$=0.966; P=0.454
Compound*Dose	$F_{2.26,13.6}$=11.9; **P=0.0008**	$F_{1.80,10.8}$=27.0; **P<0.001**	$F_{2.32,13.9}$=14.7; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=1.40; P=0.254	$F_{5,30}$=3.10; **P=0.0226**	$F_{5,30}$=0.650; P=0.664
Post Hoc	17.8 mg/kg (t=4.39, **P=0.0069**)	17.8 mg/kg (t=8.16, **P<0.001**)	17.8 mg/kg (t=5.49, **P=0.0039**)

3.2 mg/kg Yohimbine + U69,593			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.674; P=0.443	$F_{1,6}$=0.304; P=0.601	$F_{1,6}$=0.419; P=0.542
Compound	$F_{0.414,2.48}$=37.1; **P=0.0164**	$F_{0.466,2.80}$=4.81; **P=0.113**	$F_{0.406,2.44}$=0.640; P=0.345
Dose	$F_{5,30}$=6.15; **P<0.001**	$F_{5,30}$=7.96; **P<0.001**	$F_{5,30}$=1.87; P=0.130
Sex*Compound	$F_{1,6}$=7.31; **P=0.0354**	$F_{1,6}$=3.24; P=0.122	$F_{1,6}$=0.442; P=0.531
Sex*Dose	$F_{5,30}$=2.25; P=0.0754	$F_{5,30}$=1.48; P=0.227	$F_{5,30}$=1.09; P=0.389
Compound*Dose	$F_{1.73,10.4}$=10.7; **P=0.0037**	$F_{2.03,12.2}$=8.05; **P=0.0058**	$F_{2.24,13.4}$=2.85; P=0.0887
Sex*Compound*Dose	$F_{5,30}$=2.42; P=0.0585	$F_{5,30}$=0.603; P=0.670	$F_{5,30}$=0.630; P=0.678
Post Hoc	3.2 mg/kg (t=6.49, **P<0.001**) 5.6 mg/kg (t=10.1, **P<0.001**)	Not applicable	Not applicable
17.8 mg/kg Mitragynine + U69,593			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=1.79; P=0.230	$F_{1,6}$=0.176; P=0.689	$F_{1,6}$=0.0430; P=0.843
Compound	$F_{0.454,2.72}$=42.1; **P=0.0114**	$F_{0.413,2.48}$=26.1; **P=0.0244**	$F_{0.497,2.98}$=22.1; **P=0.0212**
Dose	$F_{5,30}$=21.9; **P<0.001**	$F_{5,30}$=20.9; **P<0.001**	$F_{5,30}$=6.87; **P<0.001**
Sex*Compound	$F_{1,6}$=1.17; P=0.321	$F_{1,6}$=0.104; P=0.758	$F_{1,6}$=2.41; P=0.172
Sex*Dose	$F_{5,30}$=1.34; P=0.276	$F_{5,30}$=0.561; P=0.729	$F_{5,30}$=1.66; P=0.175
Compound*Dose	$F_{2.79,16.8}$=28.7; **P<0.001**	$F_{2.15,12.9}$=21.7; **P<0.001**	$F_{2.49,15.0}$=14.0; **P<0.001**

Sex*Compound*Dose	$F_{5,30}$=1.23; P=0.319	$F_{5,30}$=0.497; P=0.776	$F_{5,30}$=0.573; P=0.720
Post Hoc	1.0 mg/kg (t=5.51, **P=0.0012**) 1.78 mg/kg (t=4.41, **P=0.0140**) 3.2 mg/kg (t=5.35, **P=0.0041**) 5.6 mg/kg (t=6.52, **P=0.0017**)	5.6 mg/kg (t=9.47, **P<0.001)**	3.2 mg/kg (t=5.33, **P<0.001**) 5.6 mg/kg (t=6.32, **P=0.0014**)
0.32 mg/kg 7-Hydroxymitragynine + U69,593			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.266; P=0.625	$F_{1,6}$=0.411; P=0.545	$F_{1,6}$=2.02; P=0.205
Compound	$F_{0.453,2.72}$=147; **P=0.0023**	$F_{0.426,2.56}$=36.7; **P=0.0155**	$F_{0.580,3.48}$=9.38; **P=0.0493**
Dose	$F_{5,30}$=37.3; **P<0.001**	$F_{5,30}$=25.9; **P<0.001**	$F_{5,30}$=8.00; **P<0.001**
Sex*Compound	$F_{1,6}$=1.28; P=0.300	$F_{1,6}$=2.42; P=0.171	$F_{1,6}$=6.63; **P=0.0421**
Sex*Dose	$F_{5,30}$=0.878; **P=0.508**	$F_{5,30}$=0.506; P=0.767	$F_{5,30}$=2.21; P=0.0798
Compound*Dose	$F_{2.70,16.2}$=47.9; **P<0.001**	$F_{2.02,12.1}$=19.9; **P=0.001**	$F_{2.59,15.6}$=5.20; **P=0.0135**
Sex*Compound*Dose	$F_{5,30}$=1.27; P=0.301	$F_{5,30}$=0.554; P=0.734	$F_{5,30}$=2.41; P=0.0598
Post Hoc	1.0 mg/kg (t=4.10, **P=0.0238**) 1.78 mg/kg (t=6.68, **P=0.0011**) 3.2 mg/kg (t=10.8, **P<0.001**)	5.6 mg/kg (t=7.22, **P<0.001)**	5.6 mg/kg (t=3.41, **P=0.0283)**

	5.6 mg/kg (t=74.7, **P<0.001**)		

Supp. Table 7 Effects of the reference Aα_2R agonists alone on food-maintained responding, antinociception, and changes in rectal temperature, as shown in **Figures 3** (lower panels), **4**, and **6**. Each sample size is four rats per sex per group. Comparisons relative to time-matching vehicle were made using a three-way repeated-measures mixed [between-subject sex and within-subject compound (compound or repeated vehicle) and compound dose] ANOVA followed by *post hoc* Bonferroni *t* tests with results shown only if there was a significant difference from the corresponding values per cycle unless noted. Statistically significant effects were shown in bold.

Lofexidine Alone (first assessment)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.175; P=0.691	$F_{1,6}$=0.937; P=0.371	$F_{1,6}$=0.123; P=0.738
Compound	$F_{0.422,2.53}$=32.8; **P=0.0181**	$F_{0.451,27.0}$=23.1; **P=0.0239**	$F_{0.403,2.42}$=73.8; **P=0.0080**
Dose	$F_{5,30}$=28.8; **P<0.001**	$F_{5,30}$=5.31; **P=0.0013**	$F_{5,30}$=60.4; **P<0.001**
Sex*Compound	$F_{1,6}$=5.03; P=0.0661	$F_{1,6}$=0.137; P=0.724	$F_{1,6}$=0.508; P=0.503
Sex*Dose	$F_{5,30}$=1.17; P=0.345	$F_{5,30}$=1.14; P=0.363	$F_{5,30}$=0.362; P=0.871
Compound*Dose	$F_{2.46,14.8}$=29.3; **P<0.001**	$F_{1.93,11.6}$=5.66; **P=0.0201**	$F_{1.69,10.2}$=51.3; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=1.56; P=0.200	$F_{5,30}$=1.08; P=0.394	$F_{5,30}$=0.423; **P=0.829**
Post Hoc	0.1 mg/kg (t=3.69, **P=0.0149**) 0.178 mg/kg (t=7.16, **P<0.001**) 0.32 mg/kg (t=7.20, **P<0.001**)	0.56 mg/kg (t=4.00, **P=0.0258**)	0.178 mg/kg (t=4.62, **P=0.0124**) 0.32 mg/kg (t=8.51, **P<0.001**) 0.56 mg/kg (t=12.4, **P<0.001**)

	0.56 mg/kg (t=9.24, **P<0.001**)		
Lofexidine Alone (reassessment)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.764; P=0.416	$F_{1,6}$=3.01; P=0.134	$F_{1,6}$=5.39; P=0.0594
Compound	$F_{0.381,2.29}$=13.4; P=0.0527	$F_{0.535,3.21}$=44.4; **P=0.0068**	$F_{0.447,2.68}$=90.5; **P=0.0045**
Dose	$F_{5,30}$=17.4; **P<0.001**	$F_{5,30}$=4.21; **P=0.0051**	$F_{5,30}$=58.8; **P<0.001**
Sex*Compound	$F_{1,6}$=1.66; P=0.245	$F_{1,6}$=3.03; P=0.132	$F_{1,6}$=5.04; P=0.0659
Sex*Dose	$F_{5,30}$=0.563; P=0.727	$F_{5,30}$=0.998; P=0.436	$F_{5,30}$=1.36; P=0.267
Compound*Dose	$F_{1.93,11.6}$=18.4; **P<0.001**	$F_{2.50,15.0}$=5.15; **P=0.0152**	$F_{2.11,12.6}$=61.5; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.905; P=0.491	$F_{5,30}$=0.829; P=0.539	$F_{5,30}$=2.91; **P=0.0295**
Post Hoc	0.178 mg/kg (t=4.73, **P=0.0019**) 0.32 mg/kg (t=6.55, **P<0.001**) 0.56 mg/kg (t=9.07, **P<0.001**)	0.178 mg/kg (t=3.57, **P=0.0375**) 0.56 mg/kg (t=3.84, **P=0.0324**)	0.1 mg/kg (t=4.41, **P=0.01117)** 0.178 mg/kg (t=5.52, **P=0.0044**) 0.32 mg/kg (t=7.79, **P<0.001**) 0.56 mg/kg (t=9.20, **P<0.001**)
1.0 mg/kg Naltrexone + Lofexidine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.0252; P=0.879	$F_{1,6}$=0.567; P=0.492	$F_{1,6}$=0.0624; P=0.811

Compound	$F_{0.515,309}$=11.5; **P=0.0442**	$F_{0.478,2.87}$=0.0156; P=0.730	$F_{0.323,1.94}$=15.4; P=0.0552
Dose	$F_{5,30}$=17.4; **P<0.001**	$F_{5,30}$=1.05; P=0.410	$F_{5,30}$=7.85; **P<0.001**
Sex*Compound	$F_{1,6}$=0.0921; P=0.772	$F_{1,6}$=0.0228; P=0.885	$F_{1,6}$=0.0586; P=0.817
Sex*Dose	$F_{5,30}$=1.95; P=0.116	$F_{5,30}$=1.09; P=0.387	$F_{5,30}$=0.108; P=0.990
Compound*Dose	$F_{1.88,11.3}$=14.7; **P<0.001**	$F_{2.66,16.0}$=1.02; P=0.401	$F_{2.22,13.3}$=22.9; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.133; P=0.984	$F_{5,30}$=1.06; P=0.400	$F_{5,30}$=0.207; P=0.957
Post Hoc	0.056 mg/kg (t=4.60, **P=0.0062**) 0.1 mg/kg (t=5.67, **P=0.0033**) 0.178 mg/kg (t=4.60, **P=0.0053**)	Not applicable	0.1 mg/kg (t=3.95, **P=0.0276**) 0.178 mg/kg (t=6.41, **P=0.0017**)
1.0 mg/kg Yohimbine + Lofexidine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.0573; P=0.819	$F_{1,6}$=4.29; P=0.0838	$F_{1,6}$=2.25; P=0.185
Compound	$F_{0.518,3.11}$=16.8; **P=0.0277**	$F_{0.249,1.49}$=0.191; P=0.387	$F_{0.262,1.58}$=42.8; **P=0.0320**
Dose	$F_{5,30}$=20.2; **P<0.001**	$F_{5,30}$=2.64; **P=0.0428**	$F_{5,30}$=15.4; **P<0.001**
Sex*Compound	$F_{1,6}$=0.108; P=0.753	$F_{1,6}$=0.324; P=0.590	$F_{1,6}$=0.389; P=0.556
Sex*Dose	$F_{5,30}$=0.462; P=0.802	$F_{5,30}$=0.497; P=0.776	$F_{5,30}$=0.293; P=0.913
Compound*Dose	$F_{2.38,14.3}$=21.7; **P<0.001**	$F_{1.47,8.82}$=3.18; P=0.100	$F_{1.89,11.3}$=20.7; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.112; P=0.989	$F_{5,30}$=1.12; P=0.372	$F_{5,30}$=0.317; P=0.899
Post Hoc	1.0 mg/kg (t=9.35, **P<0.001**)	Not applicable	1.0 mg/kg (t=4.08, **P=0.0197**)

	1.78 mg/kg (t=11.7, **P<0.001**)		1.78 mg/kg (t=8.63, **P<0.001**)
3.2 mg/kg Yohimbine + Lofexidine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.142; P=0.719	$F_{1,6}$=0.549; P=0.487	$F_{1,6}$=0.0223; P=0.886
Compound	$F_{0.580,3.48}$=14.6; P=0.028	$F_{0.441,2.65}$=0.124; P=0.540	$F_{0.441,2.64}$=4.45; P=0.123
Dose	$F_{5,30}$=25.3; **P<0.001**	$F_{5,30}$=3.35; **P=0.0160**	$F_{5,30}$=18.1; **P<0.001**
Sex*Compound	$F_{1,6}$=0.157; P=0.705	$F_{1,6}$=1.47; P=0.271	$F_{1,6}$=0.988; P=0.359
Sex*Dose	$F_{5,30}$=0.858; P=0.521	$F_{5,30}$=1.37; P=0.264	$F_{5,30}$=3.20; **P=0.0197**
Compound*Dose	$F_{1.93,11.6}$=36.9; **P<0.001**	$F_{2.20,13.2}$=3.85; P=0.0449	$F_{1.68,10.1}$=24.4; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.228; P=0.948	$F_{5,30}$=0.888; P=0.502	$F_{5,30}$=0.181; P=0.968
Post Hoc	1.78 mg/kg (t=4.33, **P=0.0042**) 3.2 mg/kg (t=6.64, **P<0.001**) 5.6 mg/kg (t=9.96, **P<0.001**)	Not applicable	5.6 mg/kg (t=5.23, **P=0.0047**)
17.8 mg/kg Mitragynine + Lofexidine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.00467; P=0.948	$F_{1,6}$=1.32; P=0.295	$F_{1,6}$=2.79; P=0.146
Compound	$F_{0.407,2.44}$=221; **P=0.0021**	$F_{0.438,2.63}$=22.9; **P=0.0254**	$F_{0.391,2.35}$=66.5; **P=0.0097**
Dose	$F_{5,30}$=55.6; **P<0.001**	$F_{5,30}$=76.8; **P<0.001**	$F_{5,30}$=20.7; **P<0.001**

Sex*Compound	$F_{1,6}$=0.0253; P=0.879	$F_{1,6}$=0.576; P=0.477	$F_{1,6}$=7.82; **P=0.0313**
Sex*Dose	$F_{5,30}$=0.860; P=0.519	$F_{5,30}$=1.72; P=0.161	$F_{5,30}$=0.623; P=0.684
Compound*Dose	$F_{2.19,13.2}$=61.9; **P<0.001**	$F_{1.99,11.9}$=97.8; **P<0.001**	$F_{2.42,14.5}$=27.5; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.749; P=0.594	$F_{5,30}$=1.92; P=0.121	$F_{5,30}$=0.320; P=0.897
Post Hoc	0.0178 mg/kg (t=4.12, **P=0.0178**) 0.032 mg/kg (t=5.65, **P=0.0035**) 0.056 mg/kg (t=10.8, **P<0.001**) 0.1 mg/kg (t=17.3, **P<0.001**) 0.178 mg/kg (t=32.2, **P<0.001**)	0.178 mg/kg (t=10.8, **P<0.001**)	0.056 mg/kg (t=4.05, **P=0.0211**) 0.1 mg/kg (t=7.42, **P<0.001**) 0.178 mg/kg (t=8.12, **P<0.001**)
0.32 mg/kg 7-Hydroxymitragynine + Lofexidine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.267; P=0.624	$F_{1,6}$=2.16; P=0.192	$F_{1,6}$=0.0466; P=0.836
Compound	$F_{0.536,3.22}$=104; **P<0.001**	$F_{0.489,2.93}$=89.8; **P=0.0033**	$F_{0.323,1.94}$=60.5; **P=0.0168**
Dose	$F_{5,30}$=121; **P<0.001**	$F_{5,30}$=43.2; **P<0.001**	$F_{5,30}$=43.2; **P<0.001**
Sex*Compound	$F_{1,6}$=0.148; P=0.714	$F_{1,6}$=4.02; P=0.0919	$F_{1,6}$=0.623; P=0.460
Sex*Dose	$F_{5,30}$=0.112; P=0.989	$F_{5,30}$=0.0424; P=0.999	$F_{5,30}$=1.74; P=0.155
Compound*Dose	$F_{2.06.12.4}$=125; **P<0.001**	$F_{2.11,12.7}$=46.8; **P<0.001**	$F_{1.48,8.89}$=54.7; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.101; P=0.991	$F_{5,30}$=0.0908; P=0.993	$F_{5,30}$=0.985; P=0.443

Post Hoc	0.056 mg/kg (t=15.9, **P<0.001**) 0.1 mg/kg (t=16.7, **P<0.001**) 0.178 mg/kg (t=34.2, **P<0.001**) 0.32 mg/kg (t=32.0, **P<0.001**) 0.56 mg/kg (t=75.3, **P<0.001**)	0.32 mg/kg (t=6.54, **P<0.001**) 0.56 mg/kg (t=12.0, **P<0.001**)	0.1 mg/kg (t=5.72, **P=0.0011**) 0.178 mg/kg (t=6.85, **P<0.001**) 0.32 mg/kg (t=8.81, **P<0.001**) 0.56 mg/kg (t=10.8, **P<0.001**)
Clonidine Alone			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.974; P=0.362	$F_{1,6}$=0.357; P=0.572	$F_{1,6}$=4.35; P=0.0822
Compound	$F_{0.443,2.66}$=54.3; **P=0.0089**	$F_{0.528,3.17}$=1.60; P=0.244	$F_{0.555,3.33}$=85.9; **P=0.0022**
Dose	$F_{5,30}$=52.0; **P<0.001**	$F_{5,30}$=3.11; **P=0.0223**	$F_{5,30}$=75.2; **P<0.001**
Sex*Compound	$F_{1,6}$=2.38; P=0.174	$F_{1,6}$=0.132; P=0.729	$F_{1,6}$=2.14; P=0.194
Sex*Dose	$F_{5,30}$=0.414; P=0.836	$F_{5,30}$=1.14; P=0.360	$F_{5,30}$=0.114; P=0.988
Compound*Dose	$F_{2.45,14.7}$=47.4; **P<0.001**	$F_{2.77,16.6}$=5.82; **P=0.0074**	$F_{2.45,14.7}$=77.3; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.555; P=0.733	$F_{5,30}$=1.28; P=0.299	$F_{5,30}$=1.23; P=0.321
Post Hoc	0.032 mg/kg (t=5.03, **P=0.0014**) 0.056 mg/kg (t=6.89, **P<0.001**)	Not applicable	0.056 mg/kg (t=5.20, **P=0.0052**) 0.1 mg/kg (t=11.5, **P<0.001**)

	0.1 mg/kg (t=7.62, **P<0.001**) 0.178 mg/kg (t=9.19, **P<0.001**)		0.178 mg/kg (t=18.6, **P<0.001**)
1.0 mg/kg Naltrexone + Clonidine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.203; P=0.668	$F_{1,6}$=0.535; P=0.492	$F_{1,6}$=0.542; P=0.490
Compound	$F_{0.377,2.26}$=17.2; **P=0.0424**	$F_{0.395,2.37}$=1.13; P=0.275	$F_{0.369,2.22}$=43.8; **P=0.0173**
Dose	$F_{5,30}$=22.8; **P<0.001**	$F_{5,30}$=2.63; **P=0.0436**	$F_{5,30}$=47.2; **P<0.001**
Sex*Compound	$F_{1,6}$=0.324; P=0.590	$F_{1,6}$=0.0269; P=0.875	$F_{1,6}$=0.653; P=0.450
Sex*Dose	$F_{5,30}$=3.35; P=0.0160	$F_{5,30}$=0.698; P=0.629	$F_{5,30}$=0.802; P=0.557
Compound*Dose	$F_{1.44,8.66}$=13.0; **P=0.0038**	$F_{1.43,8.59}$=2.27; P=0.166	$F_{1.67,10.0}$=142; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.277; P=0.922	$F_{5,30}$=0.804; P=0.556	$F_{5,30}$=0.407; P=0.840
Post Hoc	0.032 mg/kg (t=5.02, **P=0.0024**) 0.056 mg/kg (t=6.03, **P=0.0026**) 0.1 mg/kg (t=6.06, **P=0.0030**) 0.178 mg/kg (t=6.54, **P=0.0052**)	Not applicable	Vehicle (t=3.08, **P=0.0491**) 0.056 mg/kg (t=6.48, **P<0.001**) 0.1 mg/kg (t=9.55, **P<0.001**) 0.178 mg/kg (t=11.9, **P<0.001**)
1.0 mg/kg Yohimbine + Clonidine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)

Sex	$F_{1,6}$=0.128; P=0.733	$F_{1,6}$=0.0287; P=0.871	$F_{1,6}$=1.73; P=0.237
Compound	$F_{0.408,2.45}$=49.4; **P=0.0122**	$F_{0.376,2.25}$=0.251; P=0.436	$F_{0.321,1.93}$=158; **P=0.0069**
Dose	$F_{5,30}$=56.1; **P<0.001**	$F_{5,30}$=1.33; P=0.279	$F_{5,30}$=24.2; **P<0.001**
Sex*Compound	$F_{1,6}$=0.178; P=0.687	$F_{1,6}$=0.387; P=0.557	$F_{1,6}$=1.10; P=0.335
Sex*Dose	$F_{5,30}$=1.27; P=0.301	$F_{5,30}$=0.892; P=0.499	$F_{5,30}$=0.234; P=0.945
Compound*Dose	$F_{2.88,17.3}$=51.7; **P<0.001**	$F_{1.28,7.71}$=1.31; P=0.302	$F_{2.17,13.0}$=39.8; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.739; P=0.600	$F_{5,30}$=1.31; P=0.286	$F_{5,30}$=0.341; P=0.884
Post Hoc	0.1 mg/kg (t=6.31, **P<0.001**) 0.178 mg/kg (t=9.40, **P<0.001**) 0.32 mg/kg (t=8.73, **P<0.001**) 0.56 mg/kg (t=11.4, **P<0.001**)	Not applicable	0.1 mg/kg (t=3.27, **P=0.0336**) 0.178 mg/kg (t=6.19, **P<0.001**) 0.32 mg/kg (t=8.01, **P<0.001**) 0.56 mg/kg (t=14.5, **P<0.001**)
17.8 mg/kg Mitragynine + Clonidine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=28.6; **P=0.0017**	$F_{1,6}$=0.03.66; P=0.855	$F_{1,6}$=6.01; **P=0.0497**
Compound	$F_{0.551,3.31}$=1463; **P<0.001**	$F_{0.283,1.70}$=25.6; **P=0.0427**	$F_{0.275,1.65}$=305; **P=0.0062**
Dose	$F_{5,30}$=101; **P<0.001**	$F_{5,30}$=38.6; **P<0.001**	$F_{5,30}$=60.2; **P<0.001**
Sex*Compound	$F_{1,6}$=15.3; **P=0.0078**	$F_{1,6}$=0.00118; P=0.974	$F_{1,6}$=8.77; **P=0.0252**
Sex*Dose	$F_{5,30}$=3.96; **P=0.0071**	$F_{5,30}$=1.07; P=0.395	$F_{5,30}$=1.13; **P=0.366**
Compound*Dose	$F_{2.83,17.0}$=150; **P<0.001**	$F_{1.85,11.1}$=33.0; **P<0.001**	$F_{1.99,12.0}$=36.2; **P<0.001**

Sex*Compound*Dose	$F_{5,30}$=5.31; **P=0.0013**	$F_{5,30}$=0.906; P=0.490	$F_{5,30}$=0.158; P=0.976
Post Hoc	0.0056 mg/kg (t=11.7, **P<0.001**) 0.01 mg/kg (t=15.1, **P<0.001**) 0.0178 mg/kg (t=14.5, **P<0.001**) 0.032 mg/kg (t=19.1, **P<0.001**) 0.056 mg/kg (t=38.8, **P<0.001**) <u>Sex</u> 0.0056 mg/kg (t=5.05, **P<0.001**) 0.01 mg/kg (t=5.08, **P<0.001**) 0.0178 mg/kg (t=5.46, **P<0.001**) 0.032 mg/kg (t=3.77, **P<0.001**)	0.032 mg/kg (t=5.49, **P<0.01**) 0.056 mg/kg (t=6.75, **P<0.01**)	0.0178 mg/kg (t=10.3, **P<0.001**) 0.032 mg/kg (t=16.0, **P<0.001**) 0.056 mg/kg (t=20.9, **P<0.001**) <u>Sex</u> 0.0178 mg/kg (t=2.08, **P=0.056**)
0.32 mg/kg 7-Hydroxymitragynine + Clonidine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.0579; P=0.818	$F_{1,6}$=7.64; **P=0.0327**	$F_{1,6}$=17.8; **P=0.0056**
Compound	$F_{0.524,314}$=872; **P<0.001**	$F_{0.415,2.49}$=28.2; **P=0.0220**	$F_{0.538,3.23}$=116; **P=0.0016**

Dose	$F_{5,30}$=70.5; **P<0.001**	$F_{5,30}$=24.3; **P<0.001**	$F_{5,30}$=71.3; **P<0.001**
Sex*Compound	$F_{1,6}$=1.31; P=0.296	$F_{1,6}$=1.19; P=0.317	$F_{1,6}$=0.465; P=0.521
Sex*Dose	$F_{5,30}$=0.237; P=0.943	$F_{5,30}$=0.698; P=0.629	$F_{5,30}$=0.603; P=0.698
Compound*Dose	$F_{2.02,12.1}$=119; **P<0.001**	$F_{2.04,12.2}$=17.9; **P<0.001**	$F_{2.71,16.3}$=115; **P<0.001**
Sex*Compound*Dose	$F_{5,30}$=0.673; P=0.647	$F_{5,30}$=0.437; P=0.819	$F_{5,30}$=0.673; P=0.648
Post Hoc	0.0178 mg/kg (t=12.5, **P<0.001**) 0.032 mg/kg (t=15.3, **P<0.001**) 0.056 mg/kg (t=16.9, **P<0.001**) 0.1 mg/kg (t=37.7, **P<0.001**) 0.178 mg/kg (t=75.3, **P<0.001**)	0.178 mg/kg (t=7.36, **P<0.001**) <u>Sex</u> N/A	0.032 mg/kg (t=3.63, **P=0.0163**) 0.1 mg/kg (t=12.6, **P<0.001**) 0.178 mg/kg (t=15.0, **P<0.001**) <u>Sex</u> N/A

Supp. Table 8. Effects of mitragynine alone and 7-hydroxymitragynine alone on food-maintained responding, antinociception, and changes in rectal temperature, as shown in **Figures 3** (lower panels) and **5**. Each sample size is four rats per sex per group. Comparisons relative to time-matching vehicle were made using a three-way repeated-measures mixed [between-subject sex and within-subject compound (compound or repeated vehicle) and compound dose] ANOVA followed by *post hoc* Bonferroni *t* tests with results shown only if there was a significant difference from the corresponding values per cycle unless noted. Statistically significant effects were shown in bold.

Mitragynine Alone (i.p.)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.207; P=0.665	$F_{1,6}$=0.0573; P=0.819	$F_{1,6}$=0.000161; P=0.990
Compound	$F_{0.435,2.61}$=0.630; P=0.355	$F_{0.324,1.94}$=3.11; P=0.164	$F_{0.447,2.68}$=90.5; **P=0.0045**
Dose	$F_{5,30}$=6.58; **P<0.001**	$F_{5,30}$=2.94; **P=0.0284**	$F_{5,30}$=3.35; **P=0.0159**
Sex*Compound	$F_{1,6}$=1.11; P=0.334	$F_{1,6}$=0.791; P=0.408	$F_{1,6}$=0.0699; P=0.800
Sex*Dose	$F_{5,30}$=0.676; P=0.645	$F_{5,30}$=0.243; P=0.940	$F_{5,30}$=0.287; P=0.916
Compound*Dose	$F_{2.12,12.7}$=6.23; **P=0.0121**	$F_{1.82,10.9}$=2.80; P=0.108	$F_{1.79,10.7}$=3.10; P=0.0904
Sex*Compound*Dose	$F_{5,30}$=0.792; P=0.564	$F_{5,30}$=0.281; P=0.920	$F_{5,30}$=0.775; P=0.576
Post Hoc	56 mg/kg (t=3.34, **P=0.0375**)	No applicable	No applicable
Mitragynine Alone (p.o.)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=2.26; P=0.184	$F_{1,6}$=0.152; P=0.711	$F_{1,6}$=0.589; P=0.472
Compound	$F_{0.475,2.85}$=9.63; P=0.0587	$F_{0.315,1.89}$=0.161; P=0.444	$F_{0.311,1.87}$=2.07; P=0.202

Dose	$F_{5,30}$=12.3; **P<0.001**	$F_{5,30}$=3.02; **P=0.0253**	$F_{5,30}$=3.30; **P=0.173**
Sex*Compound	$F_{1,6}$=0.0593; P=0.816	$F_{1,6}$=0.0446; P=0.840	$F_{1,6}$=0.469; P=0.519
Sex*Dose	$F_{5,30}$=0.625; P=0.682	$F_{5,30}$=1.77; P=0.150	$F_{5,30}$=0.477; P=0.791
Compound*Dose	$F_{2.69,16.2}$=12.9; **P<0.001**	$F_{2.15,12.9}$=3.34; P=0.0652	$F_{1.83,11.0}$=2.65; P=0.118
Sex*Compound*Dose	$F_{5,30}$=0.842; P=0.531	$F_{5,30}$=1.00; P=0.433	$F_{5,30}$=0.826; P=0.541
Post Hoc	100 mg/kg (t=3.46, **P=0.0231**) 178 mg/kg (t=4.82, **P=0.0017**)	No applicable	17.8 mg/kg (t=3.82, **P=0.0132)** 32 mg/kg (t=4.18, **P=0.0151**) 56 mg/kg (t=3.64, **P=0.0313**)
Mitragynine Alone (s.c.)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=3.21; P=0.123	$F_{1,6}$=2.88; P=0.141	$F_{1,6}$=0.0195; P=0.894
Compound	$F_{0.438,2.63}$=1.03; P=0.293	$F_{0.430,2.58}$=4.61; P=0.121	$F_{0.355,2.13}$=4.42; P=0.131
Dose	$F_{5,30}$=10.0; **P<0.001**	$F_{5,30}$=1.57; P=0.198	$F_{5,30}$=2.40; P=0.0607
Sex*Compound	$F_{1,6}$=0.0463; P=0.837	$F_{1,6}$=3.72; P=0.564	$F_{1,6}$=0.161; P=0.702
Sex*Dose	$F_{5,30}$=1.18; P=0.341	$F_{5,30}$=0.310; P=0.903	$F_{5,30}$=0.129; P=0.985
Compound*Dose	$F_{2.57,15.4}$=8.88; **P=0.0016**	$F_{2.20,13.2}$=2.33; P=0.133	$F_{1.73,10.4}$=1.53; P=0.248
Sex*Compound*Dose	$F_{5,30}$=1.18; P=0.340	$F_{5,30}$=0.162; P=0.975	$F_{5,30}$=1.10; P=0.383
Post Hoc	178 mg/kg (t=3.91, **P=0.0104**)	No applicable	100 mg/kg (t=3.88, **P=0.0288**)
1.0 mg/kg Naltrexone + Mitragynine (i.p.)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)

Sex	$F_{1,6}$=0.00922; P=0.927	$F_{1,6}$=0.00554; P=0.943	$F_{1,6}$=0.00128; P=0.973
Compound	$F_{0.347,2.08}$=4.83; P=0.125	$F_{0.424,2.54}$=1.29; P=0.263	$F_{0.281,1.69}$=2.33; P=0.189
Dose	$F_{5,30}$=8.57; **P<0.001**	$F_{5,30}$=5.74; **P<0.001**	$F_{5,30}$=3.79; **P=0.0090**
Sex*Compound	$F_{1,6}$=0.00182; P=0.967	$F_{1,6}$=0.459; P=0.524	$F_{1,6}$=0.0193; P=0.894
Sex*Dose	$F_{5,30}$=0.747; P=0.595	$F_{5,30}$=0.256; P=0.934	$F_{5,30}$=1.79; P=0.146
Compound*Dose	$F_{2.18,13.1}$=8.27; **P=0.0042**	$F_{1.88,11.3}$=4.48; **P=0.0386**	$F_{2.14,12.9}$=9.64; **P=0.0025**
Sex*Compound*Dose	$F_{5,30}$=0.466; P=0.799	$F_{5,30}$=0.687; P=0.637	$F_{5,30}$=1.01; P=0.431
Post Hoc	56 mg/kg (t=3.98, **P=0.0101**)	56 mg/kg (t=3.71, **P=0.0279**)	56 mg/kg (t=3.89, **P=0.0255**)
3.2 mg/kg Yohimbine + Mitragynine (i.p.)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.0175; P=0.899	$F_{1,6}$=4.22; P=0.540	$F_{1,6}$=0.478; P=0.515
Compound	$F_{0.423,2.54}$=5.12; P=0.112	$F_{0.438,2.63}$=0.182; P=0.500	$F_{0.390,2.34}$=9.61; P=0.0695
Dose	$F_{5,30}$=3.30; **P=0.0172**	$F_{5,30}$=4.15; **P=0.0055**	$F_{5,30}$=1.93; P=0.120
Sex*Compound	$F_{1,6}$=0.234; P=0.646	$F_{1,6}$=1.82; P=0.226	$F_{1,6}$=0.431; P=0.536
Sex*Dose	$F_{5,30}$=0.345; P=0.881	$F_{5,30}$=1.03; P=0.420	$F_{5,30}$=4.54; **P=0.0034**
Compound*Dose	$F_{2.38,14.3}$=4.70; **P=0.0227**	$F_{1.64,9.85}$=2.75; P=0.118	$F_{1.42,8.50}$=5.37; P=0.0388
Sex*Compound*Dose	$F_{5,30}$=0.230; P=0.947	$F_{5,30}$=0.187; P=0.965	$F_{5,30}$=0.232; P=0.946
Post Hoc	56 mg/kg (t=3.89, **P=0.0123**)	Not applicable	56 mg/kg (t=5.61, **P<0.001**)
7-Hydroxymitragynine alone (i.p.)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)

Sex	$F_{1,6}$=2.99; P=0.134	$F_{1,6}$=0.677; P=0.442	$F_{1,6}$=0.337; P=0.583
Compound	$F_{0.459,2.75}$=19.0; **P=0.0292**	$F_{0.263,1.58}$=45.5; **P=0.0306**	$F_{0.635,3.81}$=0.787; P=0.364
Dose	$F_{5,30}$=30.6; **P<0.001**	$F_{5,30}$=46.2; **P<0.001**	$F_{5,30}$=2.10; P=0.0935
Sex*Compound	$F_{1,6}$=0.643; P=0.453	$F_{1,6}$=1.66; P=0.245	$F_{1,6}$=0.0726; P=0.797
Sex*Dose	$F_{5,30}$=0.705; P=0.625	$F_{5,30}$=1.63; P=0.182	$F_{5,30}$=0.794; P=0.563
Compound*Dose	$F_{2.09,12.5}$=37.6; **P<0.001**	$F_{1.37,8.25}$=41.9; **P<0.001**	$F_{3.04,18.3}$=1.92; P=0.161
Sex*Compound*Dose	$F_{5,30}$=0.864; P=0.517	$F_{5,30}$=1.66; P=0.176	$F_{5,30}$=1.04; P=0.414
Post Hoc	3.2 mg/kg (t=5.22, **P<0.001**) 10 mg/kg (t=7.47, **P<0.001**) 32 mg/kg (t=9.33, **P<0.001**)	10 mg/kg (t=5.23, **P=0.0070**) 32 mg/kg (t=10.2, **P<0.001**)	Not applicable
0.032 mg/kg Naltrexone + 7-Hydroxymitragynine (i.p.)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=26.6; P=0.633	$F_{1,6}$=2.56; P=0.161	$F_{1,6}$=0.0955; P=0.768
Compound	$F_{0.468,2.81}$=61.9; **P=0.0064**	$F_{0.525,3.15}$=31.5; **P=0.0117**	$F_{0.644,3.86}$=0.0329; P=0.757
Dose	$F_{5,30}$=55.6; **P<0.001**	$F_{5,30}$=63.1; **P<0.001**	$F_{5,30}$=1.73; P=0.0159
Sex*Compound	$F_{1,6}$=4.71; P=0.0723	$F_{1,6}$=0.243; P=0.640	$F_{1,6}$=3.28; P=0.120
Sex*Dose	$F_{5,30}$=2.69; **P=0.0398**	$F_{5,30}$=0.659; P=0.657	$F_{5,30}$=0.201; P=0.960
Compound*Dose	$F_{2.79,16.7}$=35.3; **P<0.001**	$F_{2.00,12.0}$=72.3; **P<0.001**	$F_{2.86,17.1}$=0.550; P=0.647
Sex*Compound*Dose	$F_{5,30}$=1.95; P=0.116	$F_{5,30}$=1.97; P=0.112	$F_{5,30}$=1.35; P=0.271
Post Hoc	10 mg/kg (t=5.10, **P=0.001**) 32 mg/kg (t=6.43, **P<0.001**) 56 mg/kg (t=9.00, **P<0.001**)	32 mg/kg (t=6.99, **P<0.001**)	Not applicable

		56 mg/kg (t=12.9, **P<0.001**)	
3.2 mg/kg Yohimbine + 7-Hydroxymitragynine (i.p.)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.0160; P=0.904	$F_{1,6}$=1.78; P=0.231	$F_{1,6}$=0.105; P=0.757
Compound	$F_{0.516,3.10}$=33.0; **P=0.0115**	$F_{0.408,2.45}$=14.5; P=0.0455	$F_{0.600,3.60}$=4.24; P=0.115
Dose	$F_{5,30}$=39.3; **P<0.001**	$F_{5,30}$=42.9; **P<0.001**	$F_{5,30}$=10.7; **P<0.001**
Sex*Compound	$F_{1,6}$=0.688; P=0.439	$F_{1,6}$=1.82; P=0.226	$F_{1,6}$=22.6; P=0.0032
Sex*Dose	$F_{5,30}$=1.44; P=0.240	$F_{5,30}$=0.648; P=0.665	$F_{5,30}$=1.13; P=0.368
Compound*Dose	$F_{2.24,13.5}$=51.6; **P<0.001**	$F_{1.70,10.2}$=56.5; **P<0.001**	$F_{2.16,12.9}$=8.85; **P=0.0033**
Sex*Compound*Dose	$F_{5,30}$=0.410; P=0.838	$F_{5,30}$=0.190; P=0.964	$F_{5,30}$=7.87; **P<0.001**
Post Hoc	3.2 mg/kg (t=7.38, **P<0.001**) 10 mg/kg (t=7.41, **P<0.001**) 32 mg/kg (t=10.2, **P<0.001**)	10 mg/kg (t=4.06, **P=0.0219**) 32 mg/kg (t=19.5, **P<0.001**)	3.2 mg/kg (t=3.82, **P=0.0116**) 32 mg/kg (t=3.30, **P=0.0429**)

Supp. Table 9. Pretreatment effects of naltrexone (0.032 and 1.0 mg/kg) alone, yohimbine (1.0 and 3.2 mg/kg) alone, mitragynine (17.8 mg/kg) alone, or 7-hydroxymitragynine (0.32 mg/kg) alone on food-maintained responding, antinociception, and changes in rectal temperature as shown in **Figures S3**. Each sample size is four rats per sex per group. Comparisons were made using a three-way repeated-measures mixed (between-subject sex and within-subject cycle and dose) ANOVA followed by *post hoc* Bonferroni *t* tests with results shown only if values significantly differed from those for vehicle in each corresponding cycle. Significant differences are bold.

Naltrexone			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{5,30}$=0.548; P=0.738	$F_{5,30}$=0.161; P=0.975	$F_{5,30}$=8.57; **P<0.001**
Dose	$F_{2, 12}$=0.351; P=0.711	$F_{2, 12}$=3.70; P=0.056	$F_{2, 12}$=0.842; P=0.455
Cycle	$F_{1, 6}$=0.019; P=0.894	$F_{1, 6}$=0.246; P=0.638	$F_{1, 6}$=1.40; P=0.282
Sex*Dose	$F_{10, 60}$=1.00; P=0.453	$F_{10, 60}$=2.05; **P=0.043**	$F_{10, 60}$=1.15; P=0.112
Sex*Cycle	$F_{5,30}$=0.422; P=0.829	$F_{5,30}$=0.777; P=0.574	$F_{5,30}$=0.243; P=0.940
Dose*Cycle	$F_{2, 12}$=2.13; P=0.162	$F_{2, 12}$=0.338; P=0.720	$F_{2, 12}$=0.828; P=0.460
Sex*Dose*Cycle	$F_{10,60}$=1.44; P=0.188	$F_{10,60}$=1.83; P=0.075	$F_{10,60}$=1.15; P=0.345
Post Hoc	Not applicable	Not applicable	Not applicable
Yohimbine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{5,30}$=0.922; P=0.480	$F_{5,30}$=0.513; P=0.764	$F_{5,30}$=2.69; **P=0.040**
Dose	$F_{2, 12}$=0.690; P=0.521	$F_{2, 12}$=2.53; P=0.121	$F_{2, 12}$=5.03; **P=0.026**
Cycle	$F_{1, 6}$=0.185; P=0.682	$F_{1, 6}$=2.59; P=0.159	$F_{1, 6}$=6.21; **P=0.047**
Sex*Dose	$F_{10, 60}$=1.04; P=0.419	$F_{10, 60}$=0.449; P=0.916	$F_{10, 60}$=0.968; P=0.481
Sex*Cycle	$F_{5,30}$=0.144; P=0.980	$F_{5,30}$=0.817; P=0.547	$F_{5,30}$=1.68; P=0.170

Dose*Cycle	$F_{2, 12}$=1.29; P=0.310	$F_{2, 12}$=2.73; P=0.105	$F_{2, 12}$=1.77; P=0.212
Sex*Dose*Cycle	$F_{10,60}$=1.24; P=0.285	$F_{10,60}$=1.56; P=0.140	$F_{10,60}$=2.62; **P=0.010**
Post Hoc	Not applicable	Not applicable	3.2 mg/kg (t= 5.09, **P < 0.001**) <u>Sex (3.2 mg/kg)</u> Cycle 1 (t=3.16, **P=0.009**) Cycle 2 (t=2.38, **P=0.036**)
Mitragynine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{5,30}$=0.254; P=0.935	$F_{5,30}$=1.59; P=0.194	$F_{5,30}$=1.45; P=0.237
Dose	$F_{0.422, 2.53}$=0.0492; P=0.612	$F_{0.578, 3.47}$=0.806; P=0.351	$F_{0.653, 3.92}$=2.52; P=0.178
Cycle	$F_{1, 6}$=1.72; P=0.238	$F_{1, 6}$=0.968; P=0.363	$F_{1, 6}$=1.38; P=0.285
Sex*Dose	$F_{2.49, 14.9}$=0.200; P=0.863	$F_{1.95, 11.7}$=0.370; P=0.693	$F_{2.96, 17.7}$=0.834; P=0.491
Sex*Cycle	$F_{5,30}$=0.837; P=0.534	$F_{5,30}$=1.41; P=0.250	$F_{5,30}$=1.63; P=0.182
Dose*Cycle	$F_{1, 6}$=1.54; P=0.261	$F_{1, 6}$=0.849; P=0.393	$F_{1, 6}$=0.467; P=0.520
Sex*Dose*Cycle	$F_{5,30}$=0.271; P=0.925	$F_{5,30}$=1.04; P=0.415	$F_{5,30}$=0.264; P=0.929
Post Hoc	Not applicable	Not applicable	Not applicable
7-Hydroxymitragynine			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)

Sex	$F_{5,30}$=0.0556; P=0.998	$F_{5,30}$=0.109; P=0.989	$F_{5,30}$=0.504; P=0.771
Dose	$F_{0.477, 2.86}$=0.0554; P=0.634	$F_{0.625, 3.75}$=1.40; P=0.268	$F_{0.506, 3.04}$=8.53; P=0.0630
Cycle	$F_{1, 6}$=1.74; P=0.235	$F_{1, 6}$=0.0608; P=0.813	$F_{1, 6}$=3.75; P=0.101
Sex*Dose	$F_{3.33, 20.00}$=0.0645; P=0.984	$F_{2.39, 14.3}$=1.36; P=0.292	$F_{2.30, 13.8}$=0.993; P=0.406
Sex*Cycle	$F_{5,30}$=0.253; P=0.935	$F_{5,30}$=0.228; P=0.947	$F_{5,30}$=1.24; P=0.315
Dose*Cycle	$F_{1, 6}$=1.50; P=0.266	$F_{1, 6}$=2.07; P=0.202	$F_{1, 6}$=1.07; P=0.341
Sex*Dose*Cycle	$F_{5,30}$=0.707; P=0.622	$F_{5,30}$=0.333; P=0.889	$F_{5,30}$=1.56; P=0.201
Post Hoc	Not applicable	Not applicable	Not applicable

Supp. Table 10. Effects of combinations of the reference MOR agonists with the reference Aα_2R agonists on food-maintained responding, antinociception, and changes in rectal temperature, as shown in **Figures S4 – S9**. Each sample size is four rats per sex per group. Comparisons relative to time-matching vehicle were made using a three-way repeated-measures mixed [between-subject sex and within-subject compound (compound or repeated vehicle) and cycle] ANOVA followed by *post hoc* Bonferroni *t* tests with results shown only if there was a significant difference from the corresponding values per cycle unless noted. Statistically significant effects were shown in bold.

ED_{50} Ratio (Morphine : Lofexidine = 1 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=4.14; P=0.0881	$F_{1,6}$=1.81; P=0.228	$F_{1,6}$=2.98; P=0.135
Compound	$F_{0.396,2.38}$=18.1; **P=0.0379**	$F_{0.332,1.99}$=16.1; P=0.0518	$F_{0.308,1.85}$=0.423; P=0.354
Cycle	$F_{5,30}$=36.8; **P<0.001**	$F_{5,30}$=9.26; **P<0.001**	$F_{5,30}$=3.29; **P=0.0175**
Sex*Compound	$F_{1,6}$=0.345; P=0.578	$F_{1,6}$=0.892; P=0.382	$F_{1,6}$=1.56; P=0.259
Sex*Cycle	$F_{5,30}$=4.24; **P=0.0049**	$F_{5,30}$=0.986; P=0.443	$F_{5,30}$=1.36; P=0.267
Compound*Cycle	$F_{2.25,13.5}$=38.3; **P<0.001**	$F_{1.57,9.45}$=8.80; **P=0.0092**	$F_{1.37,8,22}$=4.08; P=0.0691
Sex*Compound*Cycle	$F_{5,30}$=3.15; **P=0.0209**	$F_{5,30}$=0.680; P=0.642	$F_{5,30}$=2.15; P=0.0861
Post Hoc	Cycle 4 (t=6.37, **P<0.001**) Cycle 5 (t=7.63, **P<0.001**) Cycle 6 (t=9.33, **P<0.001**)	Cycle 6 (t=4.99, **P=0.0090**)	Not applicable
ED_{50} Ratio (Morphine : Lofexidine = 1 : 2)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)

Sex	$F_{1,6}$=8.10; **P=0.0293**	$F_{1,6}$=6.34; **P=0.0455**	$F_{1,6}$=23.2; **P=0.0030**
Compound	$F_{0.510,3.06}$=12.5; **P=0.0406**	$F_{0.295,1.77}$=15.4; P=0.0599	$F_{0.309,1.85}$=1.37; P=0.241
Cycle	$F_{5,30}$=22.9; **P<0.001**	$F_{5,30}$=5.45; **P=0.0011**	$F_{5,30}$=7.51; **P<0.001**
Sex*Compound	$F_{1,6}$=0.0751; P=0.793	$F_{1,6}$=2.27; P=0.183	$F_{1,6}$=13.0; **P=0.0113**
Sex*Cycle	$F_{5,30}$=2.03; P=0.103	$F_{5,30}$=2.24; P=0.763	$F_{5,30}$=8.25; **P<0.001**
Compound*Cycle	$F_{2.11,12.6}$=23.3; **P<0.001**	$F_{1.46,8.78}$=5.61; **P=0.0337**	$F_{1.64,9,85}$=8.94; **P=0.0078**
Sex*Compound*Cycle	$F_{5,30}$=2.41; P=0.0601	$F_{5,30}$=1.92; P=0.121	$F_{5,30}$=9.92; **P<0.001**
Post Hoc	Cycle 4 (t=3.92, **P=0.0113**) Cycle 5 (t=5.33, **P<0.001**) Cycle 6 (t=9.33, **P<0.001**) <u>Sex</u> Cycle 3 (t=2.38, **P=0.024**) Cycle 4 (t=2.68, **P=0.012**)	Not applicable <u>Sex</u> Cycle 6 (t=3.61, **P<0.001**)	Cycle 1 (t=3.14, **P=0.0468**) <u>Sex</u> Cycle 4 (t=2.50, **P=0.018**) Cycle 5 (t=4.72, **P<0.001**) Cycle 6 (t=6.44, **P<0.001**)
ED_{50} Ratio (Morphine : Lofexidine = 3 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.909; P=0.377	$F_{1,6}$=0.229; P=0.649	$F_{1,6}$=0.00112; P=0.974
Compound	$F_{0.420,2.52}$=11.7; P=0.0545	$F_{0.426,2.55}$=9.83; P=0.0634	$F_{0.286,1.72}$=0.548; P=0.321
Cycle	$F_{5,30}$=10.9; **P<0.001**	$F_{5,30}$=8.57; **P<0.001**	$F_{5,30}$=6.42; **P<0.001**
Sex*Compound	$F_{1,6}$=0.994; P=0.357	$F_{1,6}$=0.060; P=0.822	$F_{1,6}$=0.0213; P=0.889

Sex*Cycle	$F_{5,30}$=0.517; P=0.761	$F_{5,30}$=0.369; P=0.866	$F_{5,30}$=2.49; P=0.0533
Compound*Cycle	$F_{2.17,13.0}$=12.4; **P<0.001**	$F_{2.11,12.6}$=8.31; **P=0.0046**	$F_{1.34,8.06}$=6.57; **P=0.0273**
Sex*Compound*Cycle	$F_{5,30}$=0.261; P=0.931	$F_{5,30}$=0.353; P=0.876	$F_{5,30}$=2.40; P=0.0607
Post Hoc	Cycle 4 (t=3.63, **P=0.0204**) Cycle 5 (t=7.07, **P<0.001**) Cycle 6 (t=9.32, **P<0.001**)	Cycle 6 (t=4.53, **P=0.0159**)	Not applicable
ED_{50} Ratio (Morphine : Clonidine = 2 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=10.2; **P=0.0187**	$F_{1,6}$=0.348; P=0.577	$F_{1,6}$=1.52; P=0.264
Compound	$F_{0.670,4.02}$=10.6; **P=0.0348**	$F_{0.307,1.84}$=5.37; P=0.120	$F_{0.277,1.66}$=0.00850; P=0.613
Cycle	$F_{5,30}$=24.2; **P<0.001**	$F_{5,30}$=4.52; **P=0.0035**	$F_{5,30}$=2.86; **P=0.0315**
Sex*Compound	$F_{1,6}$=0.0271; P=0.875	$F_{1,6}$=0.0510; P=0.829	$F_{1,6}$=1.03; P=0.350
Sex*Cycle	$F_{5,30}$=0.756; P=0.589	$F_{5,30}$=0.105; P=0.990	$F_{5,30}$=1.01; P=0.427
Compound*Cycle	$F_{2.58,15.5}$=24.8; **P<0.001**	$F_{1.46,8.75}$=4.19; P=0.0618	$F_{1.39,8.34}$=3.11; P=0.108
Sex*Compound*Cycle	$F_{5,30}$=0.905; P=0.491	$F_{5,30}$=0.0519; P=0.998	$F_{5,30}$=1.61; P=0.188
Post Hoc	Cycle 5 (t=5.01, **P=0.0012**) Cycle 6 (t=7.61, **P<0.001**) <u>Sex</u> Cycle 3 (t=2.12, **P=0.046**)	Not applicable	Not applicable
ED_{50} Ratio (Morphine : Clonidine = 1 : 2)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)

Sex	$F_{1,6}$=2.38; P=0.174	$F_{1,6}$=2.12; P=0.195	$F_{1,6}$=1.41; P=0.279
Compound	$F_{0.560,3.36}$=21.7; **P=0.0172**	$F_{0.235,1.41}$=5.71; P=0.124	$F_{0.266,1.60}$=0.770; P=0.284
Cycle	$F_{5,30}$=45.1; **P<0.001**	$F_{5,30}$=5.72; **P<0.001**	$F_{5,30}$=7.14; **P<0.001**
Sex*Compound	$F_{1,6}$=0.606; P=0.466	$F_{1,6}$=1.61; P=0.252	$F_{1,6}$=1.25; P=0.307
Sex*Cycle	$F_{5,30}$=0.355; P=0.875	$F_{5,30}$=1.18; P=0.343	$F_{5,30}$=0.451; P=0.809
Compound*Cycle	$F_{2.69,16.1}$=46.9; **P<0.001**	$F_{1.15,6.91}$=5.45; **P=0.0492**	$F_{1.35,8.10}$=6.74; **P=0.0256**
Sex*Compound*Cycle	$F_{5,30}$=0.182; P=0.967	$F_{5,30}$=0.865; P=0.516	$F_{5,30}$=0.929; P=0.476
Post Hoc	Cycle 3 (t=3.26, **P=0.0352**) Cycle 4 (t=6.37, **P<0.001**) Cycle 5 (t=6.37, **P<0.001**) Cycle 6 (t=8.55, **P<0.001**)	Not applicable	Not applicable
ED_{50} Ratio (Morphine : Clonidine = 3 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=1.67; P=0.244	$F_{1,6}$=0.350; P=0.576	$F_{1,6}$=0.123; P=0.738
Compound	$F_{0.401,2.41}$=23.2; **P=0.0288**	$F_{0.297,1.78}$=7.36; P=0.0998	$F_{0.328,1.97}$=2.40; P=0.188
Cycle	$F_{5,30}$=25.4; **P<0.001**	$F_{5,30}$=5.30; **P=0.0013**	$F_{5,30}$=4.43; **P=0.0039**
Sex*Compound	$F_{1,6}$=1.19; P=0.318	$F_{1,6}$=0.0691; P=0.802	$F_{1,6}$=0.0365; P=0.954
Sex*Cycle	$F_{5,30}$=0.664; P=0.654	$F_{5,30}$=0.611; P=0.692	$F_{5,30}$=1.11; P=0.378
Compound*Cycle	$F_{1.80,10.8}$=34.6; **P<0.001**	$F_{1.45,8.69}$=5.12; **P=0.0416**	$F_{1.52,9.14}$=4.90; **P=0.0427**
Sex*Compound*Cycle	$F_{5,30}$=0.605; P=0.697	$F_{5,30}$=0.523; P=0.757	$F_{5,30}$=1.43; P=0.243

Post Hoc	Cycle 4 (t=6.64, **P<0.001**) Cycle 5 (t=7.81, **P<0.001**) Cycle 6 (t=9.33, **P<0.001**)	Not applicable	Cycle 3 (t=4.42, **P=0.0086**)
ED_{50} Ratio (Methadone : Lofexidine = 1 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=2.50; P=0.165	$F_{1,6}$=1.68; P=0.243	$F_{1,6}$=0.0733; P=0.796
Compound	$F_{0.311,1.87}$=6.61; P=0.105	$F_{0.268,1.61}$=4.93; P=0.131	$F_{0.287,1.72}$=0.326; P=0.367
Cycle	$F_{5,30}$=22.4; **P<0.001**	$F_{5,30}$=2.40; P=0.0605	$F_{5,30}$=2.83; **P=0.0328**
Sex*Compound	$F_{1,6}$=0.470; P=0.518	$F_{1,6}$=0.828; P=0.398	$F_{1,6}$=0.00723; P=0.935
Sex*Cycle	$F_{5,30}$=0.750; P=0.593	$F_{5,30}$=1.44; P=0.239	$F_{5,30}$=1.42; P=0.245
Compound*Cycle	$F_{1.50,9.01}$=21.4; **P<0.001**	$F_{1.37,8.22}$=2.86; P=0.123	$F_{1.29,7.75}$=3.22; P=0.107
Sex*Compound*Cycle	$F_{5,30}$=1.43; P=0.241	$F_{5,30}$=1.26; P=0.307	$F_{5,30}$=2.04; P=0.101
Post Hoc	Cycle 5 (t=3.20, **P=0.0413**) Cycle 6 (t=8.91, **P<0.001**)	Not applicable	Not applicable
ED_{50} Ratio (Methadone : Lofexidine = 1 : 2)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=5.61; P=0.0556	$F_{1,6}$=1.01; P=0.353	$F_{1,6}$=2.40; P=0.172
Compound	$F_{0.640,3.84}$=14.6; **P=0.0237**	$F_{0.223,1.34}$=4.48; P=0.141	$F_{0.335,2.01}$=1.42; P=0.242
Cycle	$F_{5,30}$=28.9; **P<0.001**	$F_{5,30}$=1.81; P=0.141	$F_{5,30}$=10.4; **P<0.001**
Sex*Compound	$F_{1,6}$=0.00743; P=0.934	$F_{1,6}$=0.311; P=0.598	$F_{1,6}$=1.65; P=0.247
Sex*Cycle	$F_{5,30}$=2.97; **P=0.0270**	$F_{5,30}$=1.87; P=0.129	$F_{5,30}$=10.8; **P<0.001**
Compound*Cycle	$F_{3.16,18.9}$=18.0; **P<0.001**	$F_{1.17,7.01}$=2.18; P=0.184	$F_{1.80,10.8}$=11.3; **P=0.0027**
Sex*Compound*Cycle	$F_{5,30}$=2.40; P=0.0604	$F_{5,30}$=1.59; P=0.193	$F_{5,30}$=11.8; **P<0.001**

Post Hoc	Cycle 5 (t=4.19, **P=0.0056**) Cycle 6 (t=7.67, **P<0.001**)	Cycle 4 (t=3.94. **P=0.0172**)	Not applicable
ED_{50} Ratio (Methadone : Lofexidine = 3 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=7.57; **P=0.0332**	$F_{1,6}$=1.04; P=0.348	$F_{1,6}$=0.129; P=0.732
Compound	$F_{0.553,3.32}$=4.19; P=0.120	$F_{0.255,1.53}$=5.08; P=0.130	$F_{0.332,1.99}$=0.459; P=0.356
Cycle	$F_{5,30}$=17.1; **P<0.001**	$F_{5,30}$=2.85; **P=0.0319**	$F_{5,30}$=2.58; **P=0.0471**
Sex*Compound	$F_{1,6}$=0.00489; P=0.947	$F_{1,6}$=0.282; P=0.615	$F_{1,6}$=0.0492; P=0.832
Sex*Cycle	$F_{5,30}$=1.13; P=0.366	$F_{5,30}$=0.356; P=0.874	$F_{5,30}$=3.41; **P=0.0147**
Compound*Cycle	$F_{2.19,13.1}$=16.3; **P<0.001**	$F_{1.28,7.67}$=3.43; P=0.0977	$F_{1.69,10.1}$=2.01; P=0.186
Sex*Compound*Cycle	$F_{5,30}$=1.25; P=0.312	$F_{5,30}$=0.312; P=0.902	$F_{5,30}$=4.13; **P=0.0057**
Post Hoc	Cycle 6 (t=6.91, **P<0.001**) <u>Sex</u> Cycle 4 (t=2.75, **P=0.011**)	Not applicable	Not applicable
ED_{50} Ratio (Methadone : Clonidine = 2 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=3.51; P=0.110	$F_{1,6}$=1.25; P=0.306	$F_{1,6}$=2.89; P=0.140
Compound	$F_{0.549,3.29}$=10.4; **P=0.0462**	$F_{0.240,1.44}$=4.83; P=0.135	$F_{0.303,1.82}$=0.00174; P=0.715
Cycle	$F_{5,30}$=24.6; **P<0.001**	$F_{5,30}$=2.86; **P=0.0316**	$F_{5,30}$=2.77; **P=0.0359**
Sex*Compound	$F_{1,6}$=0.0139; P=0.910	$F_{1,6}$=0.508; P=0.503	$F_{1,6}$=2.94; P=0.137
Sex*Cycle	$F_{5,30}$=1.26; P=0.307	$F_{5,30}$=1.36; P=0.268	$F_{5,30}$=1.16; P=0.352

Compound*Cycle	$F_{2.51,15.0}$=21.7; **P<0.001**	$F_{1.24,7.46}$=3.45; P=0.0986	$F_{1.52,9.10}$=2.86; P=0.116
Sex*Compound*Cycle	$F_{5,30}$=0.803; P=0.557	$F_{5,30}$=1.40; P=0.253	$F_{5,30}$=1.73; P=0.159
Post Hoc	Cycle 5 (t=5.12, **P=0.001**) Cycle 6 (t=6.65, **P<0.001**)	Not applicable	Not applicable
ED_{50} Ratio (Methadone : Clonidine = 1 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=2.99; P=0.135	$F_{1,6}$=2.84; P=0.143	$F_{1,6}$=1.00; P=0.356
Compound	$F_{0.511,3.06}$=10.6; **P=0.0493**	$F_{0.226,1.36}$=4.33; P=0.143	$F_{0.251,1.51}$=0.310; P=0.352
Cycle	$F_{5,30}$=18.5; **P<0.001**	$F_{5,30}$=5.24; **P=0.0014**	$F_{5,30}$=4.00; **P=0.0067**
Sex*Compound	$F_{1,6}$=0.0655; P=0.807	$F_{1,6}$=1.44; P=0.275	$F_{1,6}$=0.859; P=0.390
Sex*Cycle	$F_{5,30}$=0.476; P=0.791	$F_{5,30}$=1.32; P=0.281	$F_{5,30}$=0.775; P=0.576
Compound*Cycle	$F_{2.05,12.3}$=22.5; **P<0.001**	$F_{1.12,6.71}$=5.78; **P=0.0463**	$F_{1.25,7.49}$=4.07; P=0.0757
Sex*Compound*Cycle	$F_{5,30}$=0.368; P=0.867	$F_{5,30}$=1.09; P=0.385	$F_{5,30}$=1.18; P=0.344
Post Hoc	Cycle 5 (t=4.25, **P=0.0049**) Cycle 6 (t=7.39, **P<0.001**)	Not applicable	Not applicable
ED_{50} Ratio (Methadone : Clonidine = 4 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=2.34; P=0.651	$F_{1,6}$=5.68; P=0.0546	$F_{1,6}$=1.78; P=0.231
Compound	$F_{0.417,2.50}$=7.48; P=0.0825	$F_{0.217,1.30}$=14.9; P=0.0767	$F_{0.287,1.72}$=4.41; P=0.137
Cycle	$F_{5,30}$=14.4; **P<0.001**	$F_{5,30}$=5.06; **P=0.0018**	$F_{5,30}$=3.32; **P=0.0167**

Sex*Compound	$F_{1,6}$=0.218; P=0.657	$F_{1,6}$=2.02; P=0.205	$F_{1,6}$=1.68; P=0.243
Sex*Cycle	$F_{5,30}$=0.329; P=0.892	$F_{5,30}$=0.585; P=0.712	$F_{5,30}$=0.910; P=0.488
Compound*Cycle	$F_{2,12,12.7}$=16.5; **P<0.001**	$F_{1.16,6.99}$=5.77; **P=0.0442**	$F_{1.44,8.65}$=3.07; P=0.107
Sex*Compound*Cycle	$F_{5,30}$=0.782; P=0.571	$F_{5,30}$=0.623; P=0.683	$F_{5,30}$=1.46; P=0.233
Post Hoc	Cycle 5 (t=3.74, **P=0.0139**) Cycle 6 (t=9.33, **P<0.001**)	Not applicable	Not applicable
ED_{50} Ratio (U69,593 : Lofexidine = 1 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.953; P=0.367	$F_{1,6}$=0.129; P=0.732	$F_{1,6}$=1.41; P=0.281
Compound	$F_{0.327,1.96}$=17.3; **P=0.0495**	$F_{0.372,2.33}$=9.06; P=0.0756	$F_{0.506,3.04}$=28.4; **P=0.0147**
Cycle	$F_{5,30}$=42.4; **P<0.001**	$F_{5,30}$=6.11; **P<0.001**	$F_{5,30}$=9.92; **P<0.001**
Sex*Compound	$F_{1,6}$=1.51; P=0.265	$F_{1,6}$=0.524; P=0.496	$F_{1,6}$=1.23; P=0.310
Sex*Cycle	$F_{5,30}$=0.744; P=0.597	$F_{5,30}$=0.368; P=0.866	$F_{5,30}$=0.566; P=0.725
Compound*Cycle	$F_{2.20,13.2}$=41.3; **P<0.001**	$F_{1.87,11.2}$=7.20; **P=0.0106**	$F_{2.15.,12.9}$=12.8; **P<0.001**
Sex*Compound*Cycle	$F_{5,30}$=0.828; P=0.540	$F_{5,30}$=0.483; P=0.786	$F_{5,30}$=0.94; P=0.471
Post Hoc	Cycle 4 (t=3.96, **P=0.0089**) Cycle 5 (t=7.21, **P<0.001**) Cycle 6 (t=9.33, **P<0.001**)	Cycle 6 (t=3.85, **P=0.0366**)	Cycle 5 (t=3.65, **P=0.0456**) Cycle 6 (t=5.03, **P=0.0082**)
ED_{50} Ratio (U69,593 : Lofexidine = 1 : 2)			

Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=1.28; P=0.300	$F_{1,6}$=0.227; P=0.651	$F_{1,6}$=0.703; P=0.434
Compound	$F_{0.418,2.51}$=42.5; **P=0.0137**	$F_{0.332,1.99}$=11.2; P=0.0693	$F_{0.368,2.21}$=10.2; P=0.0689
Cycle	$F_{5,30}$=25.3; **P<0.001**	$F_{5,30}$=3.56; **P=0.0120**	$F_{5,30}$=18.1; **P<0.001**
Sex*Compound	$F_{1,6}$=1.57; P=0.257	$F_{1,6}$=1.03; P=0.350	$F_{1,6}$=0.514; P=0.500
Sex*Cycle	$F_{5,30}$=1.09; P=0.385	$F_{5,30}$=0.705; P=0.624	$F_{5,30}$=0.351; P=0.878
Compound*Cycle	$F_{2.07,12.4}$=27.7; **P<0.001**	$F_{1.99,11.9}$=4.72; **P=0.0310**	$F_{1.52,9,11}$=24.5; **P<0.001**
Sex*Compound*Cycle	$F_{5,30}$=2.34; P=0.0644	$F_{5,30}$=0.993; P=0.439	$F_{5,30}$=0.247; P=0.938
Post Hoc	Cycle 3 (t=3.28, **P=0.0328** Cycle 4 (t=3.97, **P=0.0094**) Cycle 5 (t=4.90, **P=0.0015**) Cycle 6 (t=9.24, **P<0.001**)	Not applicable	Cycle 6 (t=6.89, **P=0.0011**)
ED_{50} Ratio (U69,593 : Lofexidine = 2 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=1.14; P=0.328	$F_{1,6}$=2.17; P=0.191	$F_{1,6}$=2.65; P=0.154
Compound	$F_{0.391,2.35}$=27.6; **P=0.0250**	$F_{0.470,2.82}$=12.5; **P=0.0450**	$F_{0.275,1.65}$=5.02; P=0.129
Cycle	$F_{5,30}$=34.8; **P<0.001**	$F_{5,30}$=6.82; **P<0.001**	$F_{5,30}$=6.97; **P<0.001**
Sex*Compound	$F_{1,6}$=1.82; P=0.226	$F_{1,6}$=1.76; P=0.233	$F_{1,6}$=1.96; P=0.211
Sex*Cycle	$F_{5,30}$=0.534; P=0.749	$F_{5,30}$=0.958; P=0.459	$F_{5,30}$=2.12; P=0.0906
Compound*Cycle	$F_{2.21,13.3}$=28.1; **P<0.001**	$F_{2.42,14.5}$=7.53; **P=0.0041**	$F_{1.46,8.73}$=10.4; **P=0.0072**

Sex*Compound*Cycle	$F_{5,30}$=0.366; P=0.868	$F_{5,30}$=0.792; P=0.564	$F_{5,30}$=3.80; **P=0.0087**
Post Hoc	Cycle 4 (t=4.77, **P=0.0019**) Cycle 5 (t=7.81, **P<0.001**) Cycle 6 (t=9.32, **P<0.001**)	Not applicable	Not applicable
ED_{50} Ratio (U69,593 : Clonidine = 2 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=1.14; P=0.327	$F_{1,6}$=0.0384; P=0.851	$F_{1,6}$=4.79; P=0.0712
Compound	$F_{0.530,3.18}$=22.1; **P=0.0187**	$F_{0.420,2.52}$=9.27; P=0.0677	$F_{0.502,3.01}$=19.2; **P=0.0247**
Cycle	$F_{5,30}$=52.5; **P<0.001**	$F_{5,30}$=2.96; **P=0.0276**	$F_{5,30}$=45.7; **P<0.001**
Sex*Compound	$F_{1,6}$=1.92; P=0.216	$F_{1,6}$=0.276; P=0.618	$F_{1,6}$=3.34; P=0.117
Sex*Cycle	$F_{5,30}$=0.377; P=0.861	$F_{5,30}$=0.326; P=0.894	$F_{5,30}$=2.01; P=0.106
Compound*Cycle	$F_{2.45,14.7}$=42.3; **P<0.001**	$F_{2.25,13.5}$=3.55; P=0.0536	$F_{2.60.,15.6}$=78.3; **P<0.001**
Sex*Compound*Cycle	$F_{5,30}$=0.411; P=0.837	$F_{5,30}$=0.409; P=0.839	$F_{5,30}$=0.853; P=0.524
Post Hoc	Cycle 4 (t=6.50, **P<0.001**) Cycle 5 (t=7.51, **P<0.001**) Cycle 6 (t=9.32, **P<0.001**)	Not applicable	Cycle 4 (t=4.02, **P=0.0205**) Cycle 5 (t=4.25, **P=0.0200**) Cycle 6 (t=9.74, **P<0.001**)
ED_{50} Ratio (U69,593 : Clonidine = 1 : 2)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=1.26; P=0.305	$F_{1,6}$=0.0823; P=0.784	$F_{1,6}$=0.0124; P=0.915

Compound	$F_{0.600,3.60}$=9.83; **P=0.0447**	$F_{0.291,1.75}$=9.37; P=0.0859	$F_{0.334,2.01}$=2.61; P=0.181
Cycle	$F_{5,30}$=27.1; **P<0.001**	$F_{5,30}$=5.38; **P=0.0012**	$F_{5,30}$=10.3; **P<0.001**
Sex*Compound	$F_{1,6}$=1.34; P=0.291	$F_{1,6}$=0.0601; P=0.815	$F_{1,6}$=0.0918; P=0.772
Sex*Cycle	$F_{5,30}$=0.919; P=0.482	$F_{5,30}$=0.162; P=0.974	$F_{5,30}$=0.565; P=0.726
Compound*Cycle	$F_{2.44,14.6}$=26.4; **P<0.001**	$F_{1.58,9.46}$=5.81; **P=0.0275**	$F_{1.55,9,28}$=16.0; **P=0.0015**
Sex*Compound*Cycle	$F_{5,30}$=1.70; P=0.164	$F_{5,30}$=0.0889; P=0.993	$F_{5,30}$=0.163; P=0.974
Post Hoc	Cycle 5 (t=4.78, **P=0.0021**) Cycle 6 (t=9.13, **P<0.001**)	Not applicable	Cycle 6 (t=4.72, **P=0.0109**)
ED_{50} Ratio (U69,593 : Clonidine = 3 : 1)			
Factor	Food-Maintained Responding (%)	MPE (%)	Change in Rectal Temperature (°C)
Sex	$F_{1,6}$=0.0802; P=0.787	$F_{1,6}$=0.149; P=0.713	$F_{1,6}$=0.0109; P=0.920
Compound	$F_{0.490,2.94}$=10.2; P=0.0536	$F_{0.280,1.68}$=5.68; P=0.120	$F_{0.384,2.30}$=1.17; P=0.269
Cycle	$F_{5,30}$=57.9; **P<0.001**	$F_{5,30}$=1.19; P=0.339	$F_{5,30}$=13.3; **P<0.001**
Sex*Compound	$F_{1,6}$=3.65; P=0.105	$F_{1,6}$=1.66; P=0.245	$F_{1,6}$=0.0534; P=0.825
Sex*Cycle	$F_{5,30}$=3.68; **P=0.0103**	$F_{5,30}$=0.121; P=0.987	$F_{5,30}$=2.39; P=0.0617
Compound*Cycle	$F_{3.15,18.9}$=34.1; **P<0.001**	$F_{1.53,9.20}$=1.58; P=0.252	$F_{1.72,10.3}$=15.2; **P=0.0011**
Sex*Compound*Cycle	$F_{5,30}$=1.70; P=0.164	$F_{5,30}$=0.130; P=0.984	$F_{5,30}$=1.50; P=0.219
Post Hoc	Cycle 5 (t=4.60, **P=0.0032**) Cycle 6 (t=8.39, **P<0.001**)	Not applicable	Cycle 6 (t=4.86, **P=0.0088**)