



Published in final edited form as:

Drug Alcohol Depend. 2020 October 01; 215: 108235. doi:10.1016/j.drugalcdep.2020.108235.

Evaluation of the rewarding effects of mitragynine and 7-hydroxymitragynine in an intracranial self-stimulation procedure in male and female rats

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Abstract

Background—Kratom (*Mitragyna speciosa* Korth.) has been used in Southeast Asia for hundreds of years to increase energy, for relaxation, and to diminish opioid withdrawal. Kratom use has recently spread to Western countries. Kratom could potentially be used for the treatment of opioid withdrawal and pain, but more insight is needed into its abuse potential. Therefore, we investigated the rewarding properties of the primary kratom alkaloid mitragynine and its active metabolite 7-hydroxymitragynine, and morphine as a reference drug in male and female rats. These compounds have agonist activity at mu-opioid receptors.

Methods—The compounds were tested in an intracranial self-stimulation (ICSS) procedure, which allows for the evaluation of the rewarding/aversive and sedative effects of drugs. Rewarding

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Author Contributions

ABR, RC, and RW conducted the animal studies. TH, AS, and FL prepared the drug solutions and authenticated the compounds. LM and AB designed the studies. AB wrote the manuscript and analyzed the data. ABR, RC, RW, TH, AS, LM, and CM edited the manuscript.

Conflict of interest statement:

The authors have no conflicts of interest to disclose.

Author disclosures:

The authors have nothing to disclose.

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doses of drugs increase the brain reward thresholds, and aversive drug doses have the opposite effect.

Results—Mitragynine, 7-hydroxymitragynine, and morphine affected the brain reward thresholds. A high dose of 7-hydroxymitragynine (3.2 mg/kg) increased the brain reward thresholds, whereas an intermediate dose of morphine (10 mg/kg) decreased the reward thresholds. 7-Hydroxymitragynine and morphine affected the response latencies. Five mg/kg of morphine increased response latencies. 7-Hydroxymitragynine tended to increase the response latencies, but the post hoc analyses did not reveal a significant effect. There were no sex differences in the effects of mitragynine, 7-hydroxymitragynine, and morphine on the reward thresholds and the response latencies.

Conclusions—These initial findings indicate that mitragynine and 7-hydroxymitragynine are not rewarding in the ICSS procedure. The present results suggest that these kratom alkaloids do not have abuse potential.

Keywords

Reward; ICSS; kratom; mitragynine; 7-hydroxymitragynine; rats

1. Introduction

Mitragyna speciosa Korth., which is often referred to as kratom, has been used in Southeast Asia for centuries (Jansen and Prast, 1988). Kratom is a tropical tree that is native to countries such as Thailand, Myanmar, and Malaysia (Eisenman, 2014). Kratom use was first described in the early 1800s, and it has traditionally been used for a wide range of purposes (Jansen and Prast, 1988). In Thailand, workers used kratom to relieve fatigue and increase productivity when working outdoors. Kratom was also widely used for medical purposes and in religious ceremonies. Kratom can be consumed in many ways. Traditionally fresh Kratom leaves were chewed, or dried leaves were ground and eaten after being placed in warm water (Suwanlert, 1975). The leaves can also be smoked or used to brew tea (Singh et al., 2017).

Twenty-five alkaloids have been identified that may contribute to the physiological effects of kratom (Hassan et al., 2013). Mitragynine is the main alkaloid in kratom. The alkaloid 7-hydroxymitragynine is only a minor kratom constituent, but it is much more potent than mitragynine (Obeng et al., 2020). Mitragynine constitutes about 66 percent of the total alkaloid content and 7-hydroxymitragynine 2 percent (Hassan et al., 2013). 7-Hydroxymitragynine is also an active metabolite of mitragynine (Kruegel et al., 2019).

The kratom alkaloids act upon mu, delta, and kappa-opioid receptors. Mitragynine and 7-hydroxymitragynine act as low-efficacy agonists at mu-opioid receptors and as competitive antagonists at kappa and delta-opioid receptors (Kruegel and Grundmann, 2018). 7-Hydroxymitragynine has a tenfold higher potency at the mu-opioid receptor than mitragynine (EC_{50} values: 0.0345 ± 0.0045 and $0.339 \pm 0.178 \mu\text{M}$; G protein BRET assay) (Kruegel et al., 2016). In an in-vitro assay (guinea pig ileum preparation), 7-hydroxymitragynine had a 13 and 46-fold higher potency than morphine and mitragynine (Takayama et al., 2002). Mitragynine also inhibits radioligand binding at adenosine (A2A,

65.66 % inhibition), adrenergic (Alpha 2, 61.29 % inhibition), dopamine (D2, 54.22 % inhibition), and serotonin receptors (5-HT2C 58.77 % inhibition, 5-HT7 64.41 % inhibition) (Boyer et al., 2008). Furthermore, blockade of alpha2-adrenoceptors with idazoxan or inhibiting serotonin synthesis with p-chlorophenylalanine blocks the antinociceptive effects of mitragynine in mice (Matsumoto et al., 1996).

Kratom has complex behavioral effects in humans. At low doses, it induces stimulatory effects, and at high doses, it induces sedative, relaxing, and analgesic effects (Kruegel and Grundmann, 2018). Therefore, at low doses, kratom acts as a psychostimulant and at high doses like an opioid. In the US, kratom is used for pain relief, anxiety, depression, and opioid withdrawal (Garcia-Romeu et al., 2020). Chronic use of kratom can lead to dependence and withdrawal signs, which strongly resemble opioid withdrawal (Saingam et al., 2013; Singh et al., 2014). Kratom can also induce tolerance and dependence in rats (Harun et al., 2020).

The intracranial self-stimulation (ICSS) procedure has been widely used to study the acute rewarding effects of drugs and the aversive aspects of high doses of drugs (Barr et al., 2002; Der-Avakian and Markou, 2012). The ICSS procedure is widely used to determine if drugs have abuse potential (Negus and Miller, 2014). The rewarding effects of mitragynine and 7-hydroxymitragynine have not been evaluated in the ICSS procedure. In the ICSS procedure, rats are prepared with an electrode in the brain reward system, and the sensitivity to rewarding electrical stimuli is measured. Acute administration of low and intermediate doses of rewarding drugs increases the sensitivity to the rewarding electrical stimuli and decrease the brain reward thresholds (Esposito et al., 1978; Esposito et al., 1980; Igari et al., 2013). High and aversive doses of drugs and drug withdrawal decrease the sensitivity to rewarding electrical stimuli and increase the brain reward thresholds (Bruijnzeel et al., 2006; Cryan et al., 2003; Epping-Jordan et al., 1998). The ICSS methods can also be used to investigate the rewarding properties of opioids (Negus and Miller, 2014; Negus and Moerke, 2019). In the present study, we investigated the effects of mitragynine, 7-hydroxymitragynine, and morphine on brain reward function in male and female rats.

2. Materials and methods

2.1. Animals

Male and female Wistar rats (males 200–225 g, females 175–200 g, Charles River, Raleigh, NC) were used for this study. The rats were socially housed (2 per cage) with a rat of the same sex before the ICSS surgeries, and after the surgeries, they were singly housed. The rats were housed in a climate-controlled vivarium on a reversed 12 h light-dark cycle (light off at 7 AM). Food and water were available *ad libitum* in the home cage. The experimental protocols were approved by the University of Florida Institutional Animal Care and Use Committee.

2.2. Drugs

Salt and enantiomeric forms of the compounds used in the present study were as follows: (–)-7-hydroxymitragynine free base [synthesized from mitragynine as in (Obeng et al.,

2020)], (–)-mitragynine hydrochloride [isolated as described in (Hiranita et al., 2019)], and (–)-morphine sulfate pentahydrate (National Institute on Drug Abuse, Drug Supply Program, Rockville, MD). Doses of the compounds used in the present study are expressed as the weight of the free base or salt forms listed above. All the compounds were dissolved in a vehicle consisting of sterile water (HyClone Water for injection, Fisher Scientific, Waltham, MA) containing 5% Tween 80 (polyoxyethylene sorbitan monooleate, Fisher Scientific) and 5% propylene glycol (Sigma-Aldrich Co., St. Louis, MO). The alkaloids mitragynine and 7-hydroxymitragynine were administered intraperitoneally (IP), and morphine was administered subcutaneously (sc). Each solution was filtered through a 0.2- μ pore size syringe filter (Millex-LG, 0.20 μ m, SLLG025SS). The dose ranges of the studied compounds are based on our preliminary data and the literature (Hiranita et al., 2014; Hiranita et al., 2019; Hutchinson et al., 2012; Obeng et al., 2020).

2.3. Experimental design

The drugs were tested in drug naïve animals, and each drug was tested in a new group of animals. Mitragynine (males n=10 and females n=9; 3.2, 5.6, 10, 17.8, 32, and 56 mg/kg, IP) and 7-hydroxymitragynine (males n=12 and females n=11; 0.1, 0.32, 1, and 3.2 mg/kg, IP) were administered according to a Latin-square design. The low doses of morphine were also administered according to a Latin square design (male n=9 and females n=11; 1, 2.5, and 5 mg/kg, sc). The intermediate (10 mg/kg, sc) and a high dose of morphine (15 mg/kg, sc) were administered after the Latin square was completed). Mitragynine was administered 30 min before ICSS testing, 7-hydroxymitragynine was administered 15 min before ICSS testing, and morphine was administered 5-min before ICSS testing (Hiranita et al., 2019). There were three days between subsequent injections.

2.4. Electrode implantations and ICSS procedure

The rats were anesthetized with an isoflurane and oxygen vapor mixture and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Electrodes were implanted in the medial forebrain bundle with the incisor bar set 5 mm above the interaural line (anterior-posterior –0.5 mm, medial-lateral \pm 1.7 mm, dorsal-ventral –8.3 mm from dura)(Qi et al., 2016). After the recovery period, the rats were trained on a modified discrete-trial ICSS procedure in operant conditioning chambers (Med Associates, Georgia, VT, USA). The operant conditioning chambers were housed in sound-attenuating chambers (Med Associates, Georgia, VT, USA). The operant conditioning chambers had a 5 cm wide metal response wheel that was centered on a sidewall, and a photobeam detector recorded every 90 degrees of rotation. Brain stimulation was delivered by constant current stimulators (Model 1200C, Stimtek, Acton, MA, USA). The rats were first trained to turn the wheel on a fixed ratio 1 (FR1) schedule of reinforcement. Each quarter-turn of the wheel resulted in the delivery of a 0.5-sec train of 0.1 ms cathodal square-wave pulses at a frequency of 100 Hz. After the acquisition of responding for stimulation on this FR1 schedule, defined as 100 reinforcements within 10 min, the rats were trained on a discrete-trial current-threshold procedure. The discrete-trial current-threshold procedure is a modification of a task developed by Kornetsky and Esposito, and previously described in detail (Bruijnzeel and Markou, 2003, 2004; Kornetsky and Esposito, 1979). Each trial began with the delivery of a noncontingent electrical stimulus, followed by a 7.5 s response window during which the

animals could respond for a second identical stimulus. A response during this 7.5 s response window was labeled a positive response, while the lack of a response was labeled a negative response. During the 2 s period immediately after a positive response, additional responses had no scheduled consequences. The intertrial interval (ITI), which followed either a positive response or the end of the response window, had an average duration of 10 s (ranging from 7.5 s to 12.5 s). Responses that occurred during the ITI resulted in a 12.5 s delay of the onset of the next trial. During training on the discrete-trial procedure, the duration of the ITI and delay periods induced by time-out responses were increased until the animals performed consistently at standard test parameters. The training was completed when the animals responded correctly to more than 90% of the noncontingent electrical stimuli. It took 1 to 2 weeks of training for most rats to meet this response criterion. The rats were then tested on the current-threshold procedure in which stimulation intensities varied according to the classical psychophysical method of limits. Each test session consisted of four alternating series of descending and ascending current intensities starting with a descending sequence. Blocks of three trials were presented to the rats at a given stimulation intensity, and the intensity was altered systematically between blocks of trials by 5 μ A steps. The initial stimulus intensity was set at 30 μ A above the baseline current-threshold for each animal. Each test session typically lasted 30–40 min and provided two dependent variables for behavioral assessment (brain reward thresholds and response latencies). The brain reward threshold (μ A) was defined as the midpoint between stimulation intensities that supported responding (positive responses on at least two of the three trials) and stimulation intensities that failed to support responding (positive responses on fewer than two of the three trials for two consecutive blocks of trials). The response latency (s) was defined as the time interval between the beginning of the noncontingent stimulus and a response. A decrease in reward thresholds is indicative of an increase in reward function (Kornetsky and Esposito, 1979). Drugs with sedative effects increase the response latencies, and stimulants decrease the response latency (Igari et al., 2013; Liebman, 1985).

2.5. Statistics

The brain reward thresholds and response latencies were expressed as a percentage of pre-test day baselines. The ROUT method was used to identify outliers (Motulsky and Brown, 2006). The maximum false discovery rate (Q) was set to 0.1 percent. Three outliers were identified, and these rats were removed from the statistical analysis. The reward thresholds and response latencies were analyzed using a two-factor analysis of variance (ANOVA), with drug treatment as a within-subjects factor and sex as a between-subjects factor. A Bonferroni's post hoc test was conducted to compare drug effects to vehicle effects (Figure 4). P values that were less than 0.05 were considered significant. Significant main effects and interactions are reported in the Results section. Data were analyzed with SPSS version 25 and GraphPad Prism version 7.

3. Results

3.1. Experiment 1: Mitragynine and ICSS testing

There was an effect of mitragynine on the brain reward thresholds (Dose $F_{6,96}=4.11$, $p<0.01$; Figure 1A), and there was no sex difference in the effect of mitragynine on the reward

thresholds (Sex $F_{1,16}=1.19$, ns; Dose x Sex $F_{6,102}=0.54$, ns). Mitragynine dose and sex did not affect the response latencies (Dose $F_{6,96}=1.61$, ns; Sex $F_{1,16}=0.00$, ns; Dose x Sex $F_{6,96}=1.09$, ns; Figure 1B). There were no sex differences, and therefore the combined effects of mitragynine in the males and females are presented in figures 4A and B.

3.2. Experiment 2: 7-hydroxymitragynine and ICSS testing

Low doses of 7-hydroxymitragynine did not affect the brain reward thresholds, and a high dose increased the brain reward thresholds (Dose $F_{4,84}=9.86$, $p<0.0001$; Figure 2A). There was no sex difference in the effect of 7-hydroxymitragynine on the brain reward thresholds (Sex $F_{1,21}=2.55$, ns; Dose x Sex $F_{4,84}=9.86$, ns). A similar effect of 7-hydroxymitragynine was observed on the response latencies (Figure 2B). Low doses did not affect the response latencies, and a high dose slightly increased the response latencies (Dose $F_{4,84}=6.81$, $p<0.0001$). There was no sex difference in the effect of 7-hydroxymitragynine on the response latencies (Sex $F_{1,21}=0.86$, ns; Dose x Sex $F_{4,84}=0.63$, ns). The combined effects of 7-hydroxymitragynine in males and females are presented in figures 4C and D. The post hoc analysis shows that 3.2 mg/kg of 7-hydroxymitragynine increased the reward thresholds.

3.3. Experiment 3: Morphine and ICSS testing

Treatment with morphine affected the brain reward thresholds (Dose $F_{5,80}=4.04$, $p<0.01$; Figure 3A). The ANOVA analysis did not reveal a sex difference in the effect of morphine on the reward thresholds (Sex $F_{1,16}=2.62$, ns; Dose x Sex $F_{5,80}=1.20$, ns; Figure 3B). Morphine also affected the response latencies (Dose $F_{5,80}=3.11$, $p<0.05$). Morphine had a similar effect on the response latencies of the male and the female rats (Sex $F_{1,16}=0.00$, ns; Dose x Sex $F_{5,80}=1.32$, ns). The combined effects of morphine in the males and females are depicted in figures 4E and F. The post hoc analysis shows that 10 mg/kg of morphine decreased the reward thresholds, and 5 mg/kg of morphine increased the latencies.

4. Discussion

In the present studies, we investigated the effects of mitragynine, 7-hydroxymitragynine, and morphine on brain reward function and response latencies in male and female rats. The ANOVA analyses indicated that mitragynine, 7-hydroxymitragynine, and morphine affected the brain reward thresholds and that there was no sex difference in the effects of these drugs on the reward thresholds. The post hoc analyses indicated that 10 mg/kg of morphine lowered the brain reward thresholds and 3.2 mg/kg of 7-hydroxymitragynine increased the brain reward thresholds. A high dose of mitragynine, 56 mg/kg, tended to increase the reward thresholds, but the post hoc analyses did not reveal a significant effect. The ANOVA analysis indicated that 7-hydroxymitragynine and morphine affected the response latencies. Five mg/kg of morphine increased response latencies. 7-Hydroxymitragynine also tended to increase the response latencies, but the post hoc analyses did not reveal any significant effects. There was no sex difference in the effects of 7-hydroxymitragynine and morphine on the response latencies. These preliminary findings indicate that morphine is rewarding and 7-hydroxymitragynine aversive.

In the first experiment, we investigated the effect of mitragynine on the ICSS brain reward thresholds and the response latencies. The ANOVA analyses indicated that mitragynine affects the reward thresholds and the response latencies. A low dose of mitragynine (3.2 mg/kg, post hoc non-significant) slightly lowered the reward thresholds, and a high dose slightly increased the reward thresholds (56 mg/kg, post hoc non-significant). It is interesting to note that the effect of mitragynine on the brain reward thresholds is similar to the effects of the alkaloid nicotine. An intermediate dose of nicotine lowers the reward thresholds (0.3 mg/kg, 14 percent decrease), and a high dose increases (0.6 mg/kg, 14 percent increase) the reward thresholds (Igari et al., 2013). A low dose of mitragynine (3.2 mg/kg) induced a 13 percent decrease in the brain reward thresholds in the male rats, which is a similar decrease in thresholds, as has been observed with 0.3 mg/kg of nicotine. Several other studies have investigated the rewarding properties of mitragynine. Two studies investigated the rewarding effects of 10 mg/kg of mitragynine in a conditioned place preference procedure. In both studies, mitragynine led to the development of place preference (Yusoff et al., 2017, 2018). Mitragynine-induced place preference was blocked by the opioid receptor antagonist naloxone and the GABA_B receptor agonist baclofen (Yusoff et al., 2017, 2018). In another study, the reinforcing properties of mitragynine were investigated with an intravenous self-administration procedure. The rats were trained to self-administer methamphetamine and then switched to mitragynine, heroin, or saline (Yue et al., 2018). The rats that were switched to heroin had a higher level of operant responding than the rats that were switched to saline. However, the rats that were switched to mitragynine had a similar level of operant responding as the saline-control rats. Another self-administration study reported that rats do not acquire the self-administration of mitragynine (Hemby et al., 2019). Overall, these studies suggest that the noncontingent administration of mitragynine can be slightly rewarding under specific test conditions. Self-administration studies suggest that mitragynine has no reinforcing properties.

In the present study, we also investigated the rewarding effects of 7-hydroxymitragynine in the ICSS procedure. Low doses of 7-hydroxymitragynine did not affect the brain reward thresholds, but a high dose (3.2 mg/kg) elevated the brain reward thresholds. This suggests that a high dose of 7-hydroxymitragynine has aversive effects and impairs brain reward function. We are not aware of any other studies that investigated the rewarding effects of 7-hydroxymitragynine in the ICSS procedure or a place conditioning procedure in rats. However, a study with mice reported that 2 mg/kg induces place preference (Matsumoto et al., 2008). The effects of 7-hydroxymitragynine have been assessed in drug discrimination and self-administration procedures. One study conducted a substitution test with 7-hydroxymitragynine in rats trained to discriminate morphine (Harun et al., 2015). It was shown that 1 and 3 mg/kg of 7-hydroxymitragynine substituted for morphine. This suggests that relatively high doses of 7-hydroxymitragynine exert opioid-like effects. Rats also acquire the self-administration of 7-hydroxymitragynine (Hemby et al., 2019). It is somewhat surprising that rats self-administer 7-hydroxymitragynine, but that 7-hydroxymitragynine does not enhance brain reward function in the ICSS procedure. It is unlikely that the lack of rewarding effects was due to sedation because low doses of 7-hydroxymitragynine did not affect the response latencies. It might be possible that the ICSS procedure has a low sensitivity to determine the rewarding effects of drugs with opioid-like

effects. Not all addictive drugs enhance responding in the ICSS procedure (Wise, 1996). Although potent stimulants mostly enhance ICSS, conflicting findings have been reported with ethanol and opioids (Wise, 1996). Therefore, we also investigated the effects of morphine in our ICSS procedure.

There was a significant effect of morphine on the brain reward thresholds. Low doses did not affect the brain reward thresholds. The 10 mg/kg dose of morphine-induced a significant decrease in brain reward thresholds in the rats. Stratmann and Craft used a similar discrete trial ICSS procedure to study the effects of morphine (0.56, 1, 1.8, 3.2, and 5.6 mg/kg) on ICSS thresholds in male and female Sprague—Dawley rats (Stratmann and Craft, 1997). The low doses did not affect the brain reward thresholds and the highest dose impaired responding in half the rats. This is in line with another study that reported that 3.2 mg/kg of morphine impairs ICSS responding in drug naïve male Sprague—Dawley rats (Miller et al., 2015). However, repeated administration of the same dose facilitated ICSS responding. In contrast, it has also been reported that morphine (0.5–10 mg/kg) enhances brain reward function in a discrete trial ICSS procedure in male Brown Norway/Fischer 344 F1 (F344/BNF1) hybrid rat (Jha et al., 2004). There is evidence that the sedative effects of morphine can impair performance in the ICSS procedure. Therefore, several studies investigated the effects of a high dose of morphine, 10 mg/kg, 3.5 h after its administration using a rate-frequency curve paradigm. These studies indicate that a high dose of morphine is rewarding 3.5 h after its administration in male Sprague-Dawley and Wistar rats (Katsidoni et al., 2014; O'Neill and Todtenkopf, 2010). Interestingly, we also found that the 10 mg/kg dose of morphine is rewarding, and we observed this effect 5-min after the administration of morphine. The 10 mg/kg dose of morphine did not affect the response latencies, which indicates that this dose did not impair responding in the ICSS test. This is in line with a study that showed that 10 mg/kg does not impair locomotor activity during the first hour of the open field test in male Holtzman rats (Babbini and Davis, 1972). These findings suggest that the rewarding effects of opioids can be studied with the ICSS procedure.

In conclusion, these studies indicate that mitragynine, 7-hydroxymitragynine, and morphine affect the brain reward system. A high doses of 7-hydroxymitragynine impaired reward function and an intermediate dose of morphine (10 mg/kg) enhanced reward function. These preliminary ICSS studies suggest that the kratom alkaloids are not rewarding, and high doses might be aversive.

Acknowledgments

Funding

This work was supported by NIH grants DA47855 and DA48353.

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Highlights

- Mitragynine and 7-hydroxymitragynine are not rewarding in the ICSS procedure.
- A high dose of 7-hydroxymitragynine is aversive in the ICSS procedure.
- There are no sex differences in the effects of mitragynine and 7-hydroxymitragynine.
- Morphine is rewarding in the ICSS procedure.

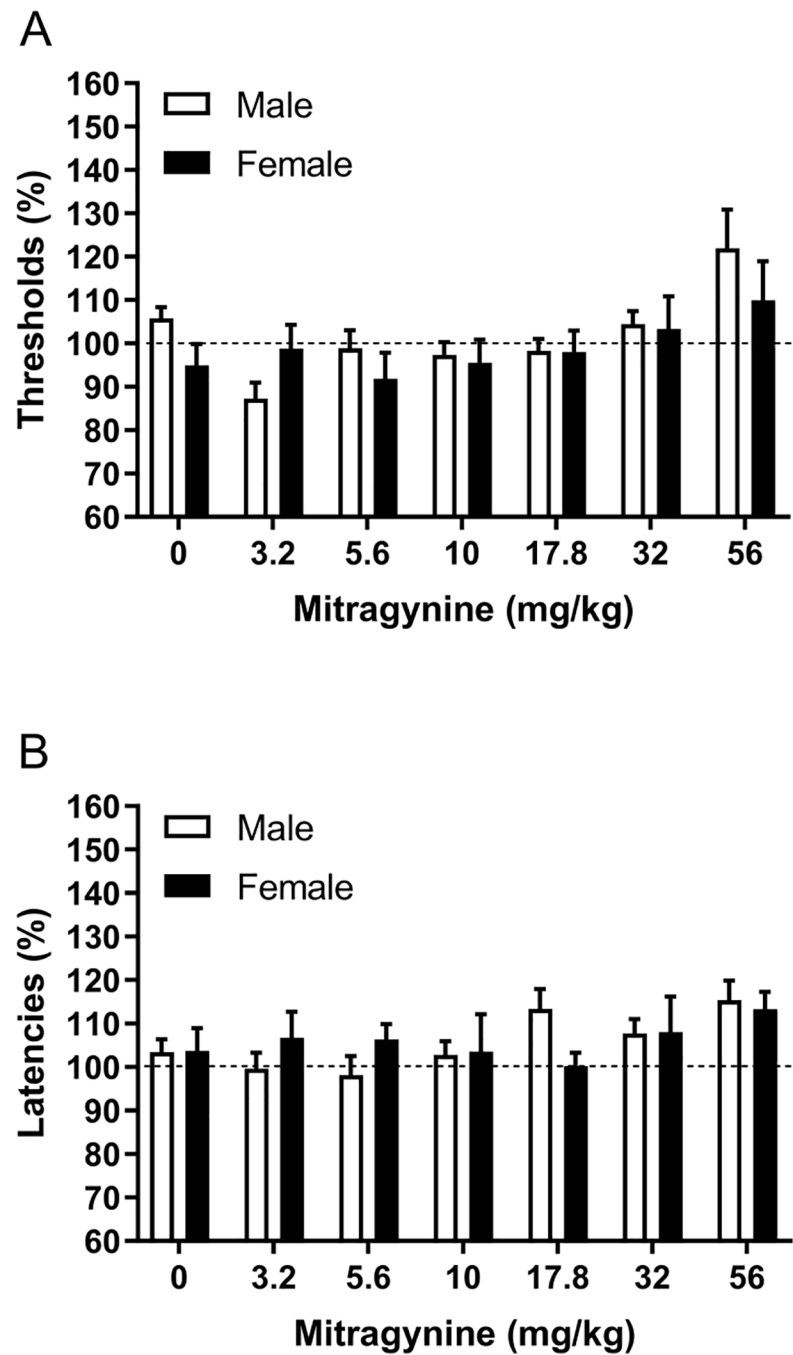


Figure 1. Effects of mitragynine on brain reward thresholds and response latencies in males and females.

The figures show the effect of mitragynine on the reward thresholds (A) and response latencies (B). Males, N=10; Females, N=8. Data are expressed as means \pm SEM.

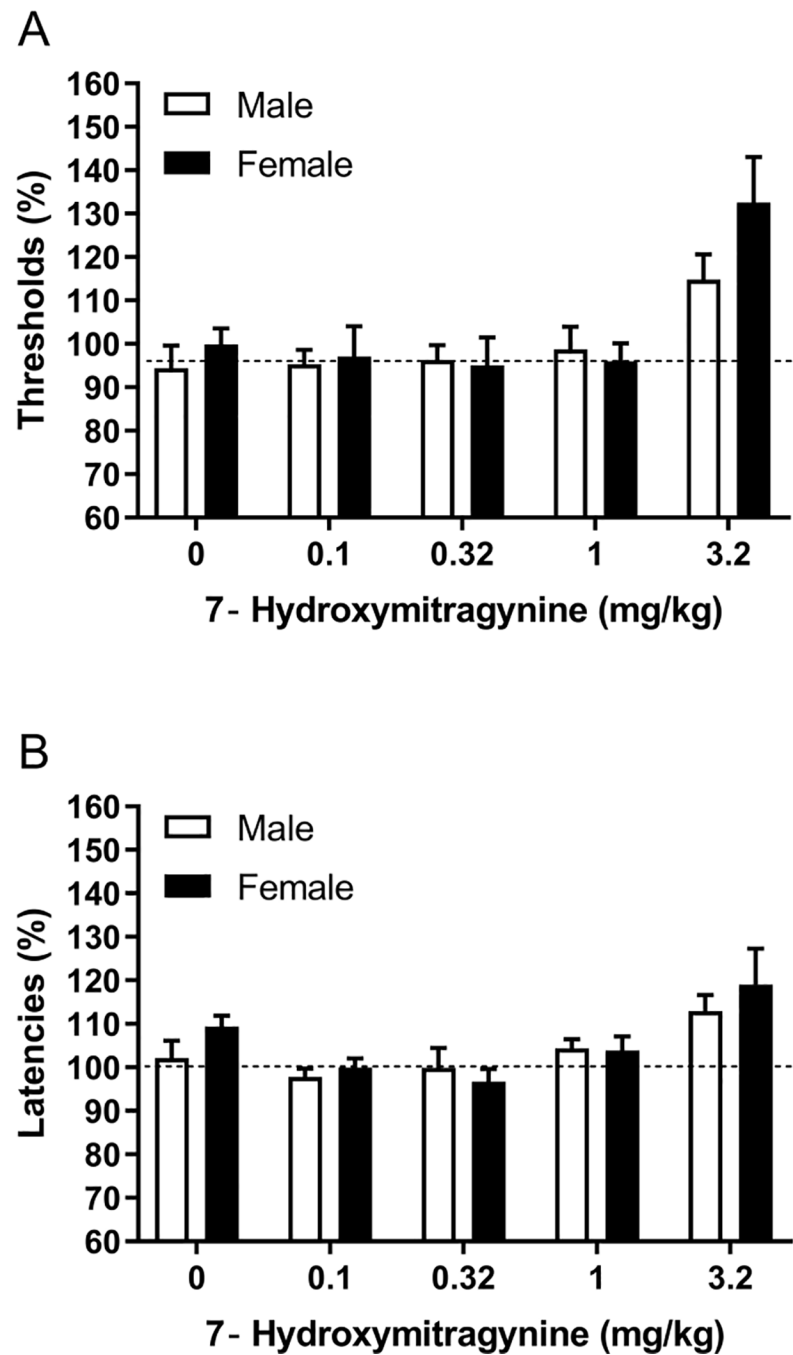


Figure 2. Effects of 7-hydroxymitragynine on brain reward thresholds and response latencies in males and females.

The figures show the effect of 7-hydroxymitragynine on the reward thresholds (A) and response latencies (B). Males, N=12; Females, N=11. Data are expressed as means \pm SEM.

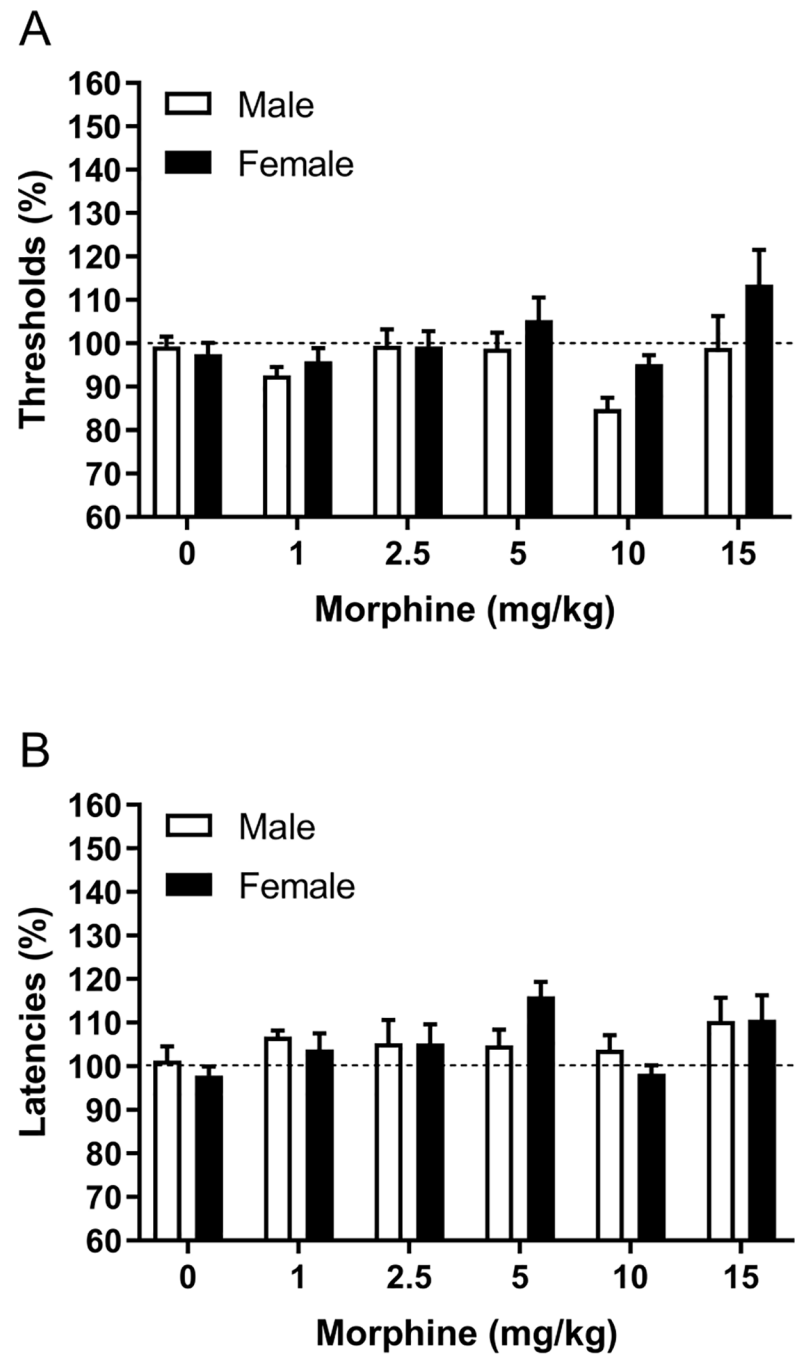


Figure 3. Effects of morphine on brain reward thresholds and response latencies in males and females.

The figures show the effect of morphine on the reward thresholds (A) and response latencies (B). Males, N=8; Females, N=10. Data are expressed as means \pm SEM.

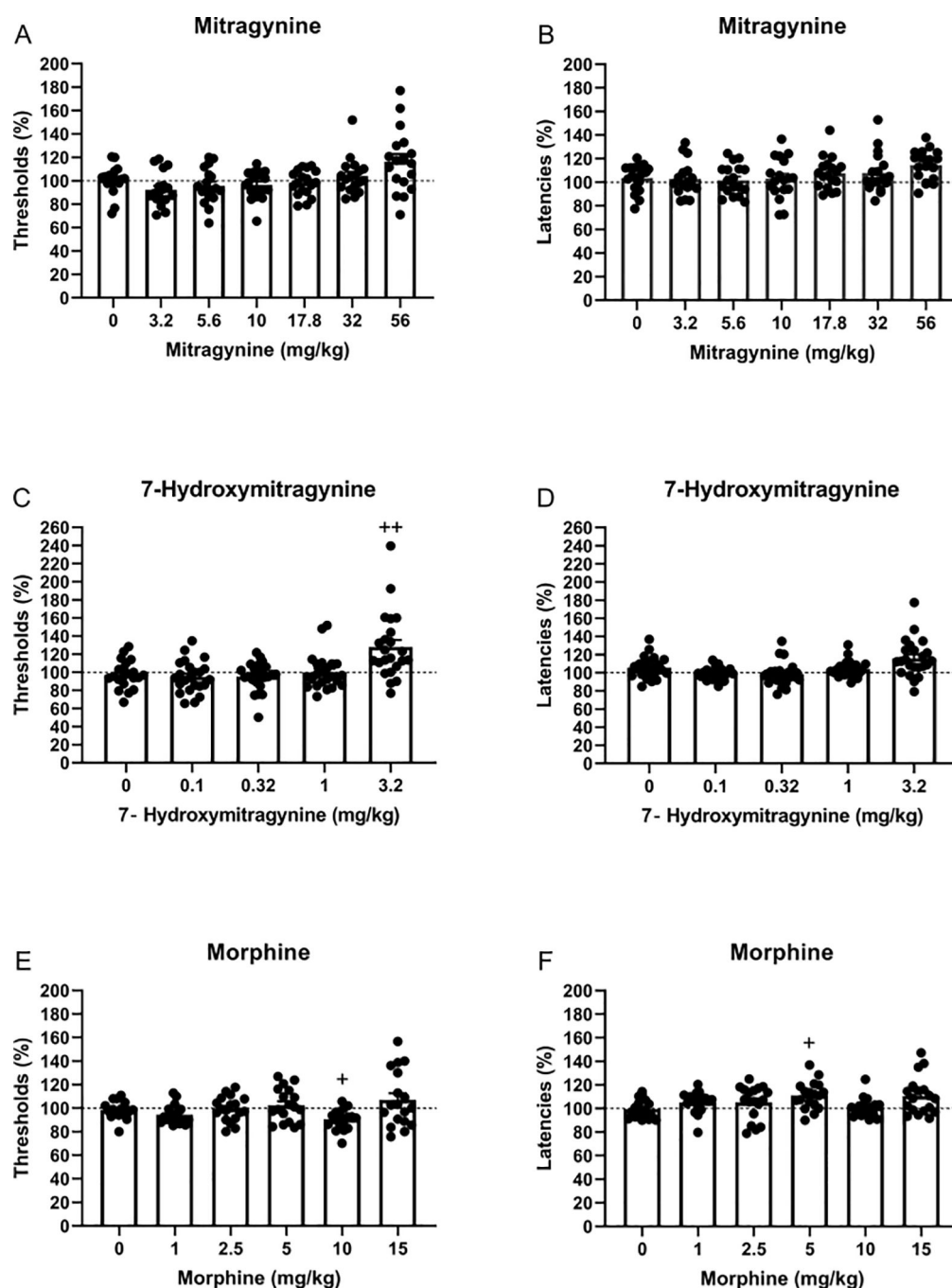


Figure 4. Effects of mitragynine, 7-hydroxymitragynine, and morphine on brain reward thresholds and response latencies.

The figures show the effect of mitragynine (A, B), 7-hydroxymitragynine (C, D), and morphine (E, F) on the reward thresholds and latencies for both males and females combined. Plus signs indicate an increase or decrease in the reward thresholds or response latencies compared to rats treated with vehicle. +, $p < 0.05$. ++, $p < 0.01$. A,B: $N = 18$; C,D: $N = 23$; E,F: $N = 18$. Data are expressed as means \pm SEM.