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Examining the Effects of Kratom Alkaloids on Mesolimbic Dopamine Release

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EXAMINING THE EFFECTS OF KRATOM ALKALOIDS ON MESOLIMBIC DOPAMINE

RELEASE

by

James Paul Manus

A Thesis

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Abstract

Kratom, derived from the plant Mitragyna speciosa, is reported to produce stimulant-like effects at low doses and opiate-like effects at high doses. Mitragynine (MG) and 7-hydroxymitragynine (7-HMG) are the major psychoactive constituents of kratom, but the neural mechanisms of these alkaloids are not clear. Given that the effects of kratom are often compared to those of drugs with a high abuse liability, the current study aimed to determine the effect of MG and 7-HMG on reward-related neurotransmission. In vivo fixed potential amperometry was used to quantify stimulation-evoked phasic dopamine release in the nucleus accumbens (NAc) of anesthetized male and female mice. During dopamine recordings, mice received an i.p. injection of either MG (1, 15, or 30 mg/kg i.p.), 7-HMG (0.5, 1, or 2 mg/kg i.p.), or vehicle, and dopamine recordings continued for 90 min. Using specific stimulation parameters, dopamine autoreceptor functioning was measured 30 min post injection. Sex effects were observed following MG but not 7-HMG. In males, there was a significant time x MG dose interaction with the low dose seeming to increase dopamine release relative to vehicle and the higher doses towards the end of the recording period. However, follow-up tests revealed no significant dopamine release differences between doses at specific time points. Low dose MG also increased dopamine autoreceptor functioning (+50% from vehicle) in males. Regarding 7-HMG, both sexes responded similarly with a significant time x 7-HMG dose interaction effect on dopamine release. Follow-up tests revealed significant differences between 7-HMG doses from 60-90 min post injection, with the low dose increasing dopamine release (+18% from vehicle) compared to the high dose (-22% from vehicle). 7-HMG did not alter dopamine autoreceptor functioning. Neither MG nor 7-HMG altered the clearance rate of stimulation-evoked dopamine, indicating no drug effect on dopamine transporter functioning. In conclusion, dose effects were observed following both MG and 7-HMG. The low dose of MG (1 mg/kg) affected dopamine release in males more than females, potentially through actions on dopamine autoreceptors, and in both sexes the low dose of 7-HMG (0.5 mg/kg) increased dopamine release while the high dose (2 mg/kg) had the opposite effect. Although these increases in dopamine release are considerably less than those normally observed in stimulants with a high abuse liability, these findings do suggest that MG and 7-HMG alter dopamine transmission. An increased understanding of the neural mechanisms of kratom may provide insight on its potential uses and abuse liability.

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Background and History of Kratom

Kratom (*Mitragyna speciosa*) is a plant native to Southeast Asia that has been consumed by indigenous cultures for hundreds of years for its complex stimulant, analgesic, and medicinal effects in the form of capsules or tea-like brews (Ahmad et al. 2012). Within the last decade, the awareness and availability of kratom has shown a marked increase in the United States (Anwar and Law, 2016). The American Kratom Association is currently one of the few sources that have population estimations available. Based on these estimations from the AKA (AKA, 2020), in 2016 the United States imported approximately 4.1 million pounds of kratom material from Indonesia, one of the world's largest exporters of kratom. By considering the amount of imported material that is manufactured into various kratom products and the average consumer use/dosage, in 2016 there were an estimated 5.1 million kratom consumers in the United States (AKA, 2020). In 2020, these numbers were greatly increased with approximately 10.2 million pounds of kratom material imported to the United States and an estimated 12.9 million kratom consumers. However, when accounting for the volume of direct sales from Indonesian farmers to U.S. consumers and small vendors, the estimated number of current kratom consumers is even higher, at 15 million consumers (AKA, 2020). Other researchers have estimated there to be 10-16 million people in the United States that use kratom for diverse health reasons (Henningfield, 2019). Furthermore, by using the IMPLAN model based on employment data from the U.S. Bureau of Economic Analysis, in 2020 the kratom sector accounted for 18,560 jobs, $882,800,00 in labor income, and contributed a total of $1,368,576,000 to the U.S. GDP (AKA, 2020), thus, signifying the extent of the kratom consumer market and its establishment in the United States.

Although, the data is not yet clear if these reported numbers were derived exclusively from kratom sales or were influenced by the presence of integrated business models. The amount of scientific literature surrounding kratom is expanding but not yet at a rate that mirrors the rise of its consumption, and many of kratom's neural mechanisms are still not fully understood. Specifically, the effect kratom has on neural pathways related to reward processing has not yet been sufficiently explored.

Historically, the plant has a tumultuous past regarding regulation and legality. Most notably, the Thai government passed the Kratom Act 2486, effective August 3, 1943, which made planting the tree illegal. This was in response to a rise in kratom use when opium became very expensive in Thailand (which the government was profiting from in the form of sanctioned opium dens with inflated prices) and the Thai government was attempting to gain control of the opium market (Meireles, 2019). Locals were most commonly using kratom to wean themselves off their opium dependence and as a stimulant to help manage the painful demands of physical labor (Veltri and Grundmann, 2019). In 2018, the Thai government legalized the production, import, export, possession and use of kratom products for medicinal purposes. The distribution of kratom has consequently grown. Regarding the US, there are currently 6 states that have labelled kratom as a controlled substance (Alabama, Arkansas, Indiana, Rhode Island, Vermont, and Wisconsin) (Veltri and Grundmann, 2019). In 2014, synthetic kratom was labelled illegal in TN, but according to the current legislation, kratom powder, kratom capsules containing pure kratom powder, and kratom extracts are legal in TN for those over the age of 21 (Williams and Nikitin, 2020). In 2017, the US Drug Enforcement Administration identified kratom as a Drug of Concern but received substantial pushback from the kratom user community who touted its benefits (DEA, 2017). As such, kratom is currently legal in most states and is distributed and/or

purchased at smoke shops**,** convenience stores, or online in the form of powder, capsules, extracts, or is consumed in a tea-like brew (Swogger et al., 2015).

Use of Kratom

"Kratom" is typically consumed as a tea-like brew, commercially prepared liquid, or in the form of capsules (Henningfield et al., 2017). *Mitragyna speciose* is a member of the Rubiaceae family, which also includes the genus Coffea (Eisenman, 2015). Similar to coffee, kratom has been traditionally used in regions of Southeast Asia as a stimulant to combat fatigue and improve work productivity, but also to manage pain and opioid withdrawal (Suwanlert, 1975). In addition, kratom preparations have also been used for centuries during socioreligious ceremonies and to treat various medical conditions such as diarrhea, pain, coughing, or to ameliorate withdrawal symptoms from other addictive drugs (Ahmad et al. 2012) (Assanangkornchai, 2007). Inversely in the early 2000s, a cocktail consisting of boiled kratom leaves, cola soft drink, codeine cough syrup, and ice (or another 4th ingredient) known as "4x100" became a popular drink and rising social problem among the youth in Thailand (Davidson et al., 2020). Kratom is most commonly used without a prescription across cultures to help wean opioid dependence, relieve pain, stimulant purposes, and in some cases recreation (Boyer et al., 2008) (Davidson et al., 2020). In a 2017 U.S. study detailing kratom user demographics and use patterns, Garcia-Romeu et al. (2020) found that 91% of their 2,798 respondents in a cross-sectional, anonymous online survey used kratom for pain relief. The researchers also found most respondents used kratom to help treat mood-related issues like anxiety (67.2%) or depression (64.5%) and to treat opioid withdrawal symptoms (87.3%) (Garcia-Romeu et al., 2020). Most of these respondents reported using kratom within the past

day (80.7%), more than 100 times in total (76.2%), and continued daily use (59.1%) (Garcia-Romeu et al., 2020).

Neuropharmacology of Kratom

The neural mechanisms of kratom are not yet understood. Containing more than 3 dozen indole alkaloids, the psychostimulant and opiate-like effects of kratom are in part attributed to its two primary alkaloids mitragynine (MG) and its metabolite 7-hydroxymitragynine (7-HMG) that are exclusive to *Mitragyna speciosa* (Johnson et al., 2020). MG is an indole-based partial agonist of the mu-opioid receptor and the most abundant (accounting for up to 66% of the alkaloid content) psychoactive alkaloid in the plant *Mitragyna speciose* (Matsumoto et al., 1996). 7-HMG, a major metabolite of MG, while present in the plant in much smaller quantities (2% of the alkaloid content), is noted in numerous studies to be a much more potent opioid agonist and analgesic (Harun et al., 2015; Matsumoto et. al., 2004; Ponglux et al.,1994). While both MG and 7-HMG both target opioid receptors they differ in their binding affinities (Prozialeck et al., 2012). In comparative studies, MG has been shown to have less affinity for opioid receptors than morphine, but 7-HMG has been shown to be approximately 46 times more potent than MG and 13 times more potent than morphine (Matsumoto et al., 2006). Currently, the exact mechanisms in which kratom alkaloids act at each of the receptors are still being discovered (Matsumoto et. al., 2004). Kruegel et. al. (2016) reported that MG and 7-HMG seem to have negligible effects on κ-receptors, although they can act as partial agonists at μ-receptors and competitive antagonists δ-receptors. While the physiological significance and precise interactions of kratom's alkaloids are still largely unclear, they have been shown to bear at least partial affinity for 5-HT2C and 5-HT7 serotonin receptors, D2 dopamine receptors, and A2A adenosine receptors

(Matsumoto et. al., 2005). Notably, because the alkaloids in kratom produce similar but nonidentical effects that are distinct from other opioids both structurally and pharmacodynamically, these compounds have been called "nature's first" atypical opioids to properly distinguish them from traditional opioids. (Raffa, Pergolizzi, Taylor, and Ossipov, 2018). Furthermore, while the understanding of kratom's unique pharmacology is still developing, it has been postulated that MG is converted to 7-HMG in the liver via hepatic metabolism (Yusof et. al., 2019). Moreover, there is also emerging evidence suggesting that 7-HMG is stable in plasma using rodent and primate models but is subsequently converted to mitragynine pseudoindoxyl (an even more potent opioid) in human plasma (Kamble et al., 2020). Consequently, this has led some groups to hypothesize that 7-HMG represents the active metabolite of MG and 7-HMG accounts for the effects that have historically been attributed to its precursor MG (Kruegel et. al., 2019; Yusof et. al., 2019). These studies also posit this endogenous generation of 7-HMG from MG results in any ingested amount of 7-HMG present in kratom products to be relatively inconsequential (Kruegel et. al., 2019; Yusof et. al., 2019). The relevance of this discovery has not yet been confirmed in human physiology as current studies are still utilizing animal models.

Abuse Liability of Kratom

Given the rise in consumption and the neuropharmacological overlap with opioids, researchers have begun to investigate the potential abuse liability of kratom. To date, there is a lack of controlled studies and characterization of kratom's abuse liability in humans (Garcia-Romeu et al., 2020). Regarding the available clinical findings, there are some related case reports suggesting kratom use may lead to withdrawal symptoms (irritability, dysphoria, nausea,

hypertension, insomnia, yawning, rhinorrhea, myalgia, diarrhea, and arthralgias) (Prozialeck et al., 2012; Suwanlert, 1975; Trakulsrichai et al., 2013), overdose (Kronstrand et al., 2011), and the development of tolerance and/or increased intake over time (Hassan et al., 2013). It should be noted that in many case reports, especially those resulting in death, the negative effects of kratom are often correlated with compounding substance abuse and mental illness (Swogger et al., 2018), meaning that clinical kratom withdrawal symptoms are difficult to separate from users' opioid or other substance withdrawals (Assanangkornchai et al., 2007; Suwanlert, 1975). In a CDC report from July 2017-December 2017 of 27,338 overdose deaths, 152(<1%) decedents tested positive for "kratom-involved" on postmortem toxicology (Olsen et al., 2019). Of these 152, kratom was found to be the only substance in 7 decedents, and the researchers report the presence of additional substances cannot be ruled out (Olsen et al., 2019). Other drugs associated with these overdoses were fentanyl and its analogs (65.1%), heroin (32.9%), benzodiazepines (22.4%), prescription opioids (19.7%), and cocaine (18.4%) (Olsen et al., 2019). Additionally, while not mentioned in this CDC report, alcohol contributes to approximately 22.1% of overdose deaths related to prescription opioids (Jones, Paulozzi, and Mack, 2010) and the comparative contribution of kratom to this crisis is still developing. However, a cross-sectional survey of 293 male regular kratom users (regular user - for at least 6 months) (mean age – 28.9 years old) conducted in Malaysia found those who used kratom more than 3 times a day were 5.19 times more likely to report severe kratom dependence (Singh and Muller, 2014). Furthermore, within this sample of users who abruptly abstained from kratom use researchers found 65% of these users experienced mild withdrawal symptoms, 35% experienced moderate to severe effects, and 64% of these withdrawal symptoms lasted for 1-3 days (Singh and Muller, 2014). Although, the authors of this study do not clarify what constitutes mild, moderate, or severe effects, some

withdrawal symptoms experienced by kratom users were: difficulty sleeping, decreased appetite, nausea, vomiting, muscle spasms, abdominal pain, sweating, shakiness or tremors, aggression, and hostility (Singh and Muller, 2014). Body aches, cramps, and severe muscle pain were also reported by 76% of users who abruptly abstained (Singh and Muller, 2014). Results from the previously mentioned online survey by Garcia-Romeu et al. (2020) also found 9.5% of their 2,798 respondents reported kratom-related withdrawal symptoms. Despite these findings, based on the DSM-V symptom checklist, 87.7% of respondents did not meet the criteria necessary for a kratom-related substance use disorder (Garcia-Romeu et al., 2020).

Animal studies have revealed more specific characteristics of the potential abuse liability of kratom by examining dose-dependent effects of the kratom alkaloids MG and 7-HMG. Rats have been shown to respond in a dose-dependent manner when assessing locomotor and reward-related behaviors after acute MG administration (Yusoff et. al., 2016). In this rodent study, researchers found that varying doses of MG (1, 10, and 30 mg/kg, ip) induced significant differences in locomotor activity, rearing behavior, and conditioned place preference. The low dose induced profound hyperlocomotion and rearing behavior compared with vehicle group, while the medium and high dose significantly reduce the total distance travelled and number of rears. Yusoff et. al. (2016) found the behavioral effects of MG at their largest dose (30 mg/kg) to be similar to the effects of morphine as well as methamphetamine. Following the cessation of MG treatment (30 mg/kg, ip) for 14 days, severe somatic withdrawal symptoms (e.g., paw tremor, body tremor, wet-dog shakes, ptosis, piloerection, teeth chattering and grooming) developed after 12 hours and elevated levels of anxiety (significant decline in open arm time) were evident after 24 and 48 hours (Yusoff et al., 2016). However, these somatic and anxiolytic signs of withdrawal were reversed or disappeared after 72 hours, suggesting to the researchers

that MG withdrawal effects could have a restricted period of 48 hours (Yusoff et al., 2016).

Similarly, Hazim et al. (2011) showed that acute MG induces locomotor activation at a low dose but not at high doses in rodents. However, behavioral results surrounding the reinforcing effects of MG are not all congruent. For example, MG has been shown to induce a conditioned place preference for rats in doses of 10 mg/kg and 30 mg/kg, with the MG preference being similar in magnitude to that of morphine and methamphetamine (Yusoff et al., 2016). On the contrary, Hemby et al. (2018) found that none of their doses of MG (25, 50, 100 and 150 μg/inf) were self-administered any differently than saline. Drug self-administration is a critical behavioral paradigm for establishing/understanding abuse liability (O'Connor et al., 2011; Collins et al., 1984). Furthermore, MG was not reliably self-administered when substituted for morphine, but MG exposure at a low dose over a 2-week period significantly reduced morphine self-administration up to 3 days following the last exposure (Hemby et al., 2018). Researchers from this study also report their findings pertaining to MG are consistent with anecdotal reports describing kratom use and a reduction in opiate intake (Hemby et al., 2018). Similarly, in rats trained to self-administer methamphetamine, MG pretreatment (0.1 to 3.0 mg/kg) reduced response rates maintained by heroin (Yue, Kopajtic, & Katz, 2018). Overall, more research is needed on the rewarding value of MG.

Although less research has been conducted on 7-HMG, some researchers are suggesting it may be more rewarding than MG, and that kratom distributors in the United States are altering concentrations of 7-HMG for these desired effects (Lydecker et al., 2016). Notably, the current lack of regulation, non-standardization, and possible contamination or adulteration of kratom products poses perhaps the most significant risks to public health (Prozialeck et al., 2020). 7-HMG has been shown to increase locomotor activity and produce CPP in rodents at 2 and 4

mg/kg (Matsumoto et al., 2006; Matsumoto et al., 2008). Furthermore, self-administration of 7-HMG was established in drug-naive rats and when substituted for morphine (Hemby et al., 2018). Furthermore, 7-HMG self-administration exhibited an inverted 'U' dose–effect curve at 5 and 10 μg/inf, a pattern typically associated with drugs of abuse where high doses decrease responding, potentially through aversion or motor impairment, and low to moderate doses increase responding (Wilson, Hitomi, & Schuster 1971; Pickens 1978; Hemby et al. 2018). While there is scarce research on 7-HMG and withdrawal symptoms, Hemby et al. (2018) found that rats displayed an increased morphine intake after administrations of 7-HMG but no physical signs of withdrawal (e.g., excretion of feces and urine, salivation, behavioral jumping, wet dog shakes). Increased morphine intake following kratom exposure could be a compensatory response to withdrawal from 7-HMG or an indicator that 7-HMG has significant abuse potential (Hemby et al., 2018). Although 7-HMG only constitutes 2% of the alkaloid content in kratom, the concentration is sometimes higher in commercial products (Lydecker et al., 2016) highlighting the need to further investigate this alkaloid.

The addictive properties of most drugs of abuse are driven by their ability to increase activity of the mesolimbic dopamine pathway, which consists of dopamine cell bodies located in the ventral tegmental area (VTA) that project to the limbic system, most notably the nucleus accumbens (NAc) (Koob & Bloom, 1988; Wise, 2005). In freely moving rats, dopamine neurons fire tonically at ~4hz and burst fire phasically at ~20hz (Hyland et al., 2002). Tonic firing is suggested to occur when no behaviorally relevant stimuli are present and produces low concentrations of extracellular dopamine (Goto et al., 2007). Conversely, phasic firing occurs in relation to behaviorally significant external stimuli whose detection is crucial for learning (Schultz et al., 1993). This mode of firing is thought to evoke a large enough extracellular

concentration for the highlighting of salient stimuli (Middleton, 2000). To date, no studies have examined the effects of kratom or the alkaloids MG and 7-HMG on phasic dopamine release, and only one study related to tonic dopamine. Vijeepallum et al. (2019) found that a low dose of the methanolic extract from the kratom leaf did not alter dopamine levels, as measured in dissected brain regions with ELISA, but did attenuate ethanol-induced increases in dopamine levels. Kratom has been shown to bind to dopamine D2 receptors (Boyer et al., 2008) while also binding at low affinity to D1 receptors (Stolt et al., 2014). Repeated administration of MG has been shown to decrease D2 and increase DAT sites in the mesencephalon, which houses the VTA, but not in the NAc (Yusoff et al., 2014). Altogether these data suggest that kratom alters dopamine functioning, but whether this dopaminergic effect results in the increased dopamine activity levels associated with drugs of abuse is not clear.

Furthermore, the United States continues to struggle with the ongoing opioid epidemic, even during the current pandemic. Historically, women have been prescribed opioids for the treatment of acute and chronic pain in significantly greater numbers than men (Goetz, Becker, and Mazure, 2021). The use of these prescription medications has been thought to be the primary precursor for misuse and addiction to opioids for women (Goetz, Becker, and Mazure, 2021). While the exact levels of women's exposure and resultant consequences have been limited, further research could be used to increase our understanding of the underlying biological and psychosocial differences among men and women (Goetz, Becker, and Mazure, 2021). In animal models, female rodents have been shown to exhibit more rapid acquisition and escalation of drug taking, higher motivation for drugs of abuse, and more like to experience relapse-like behaviors when compared to males (Becker, 2016). Becker and Chartoff (2019) make a crucial distinction that there is evidence that the qualitative aspects of the addiction phases do not differ

significantly between the males and females, but one sex might be more likely to exhibit a trait when compared to the other which then results in population differences. However, heroin self-administration has been found to be lower in males than females and are presumed to vary based on variations in circulating ovarian hormones (Lacy, Strickland, Feinstein, Robinson, and Smith, 2016). Additionally, female rats have been shown to be more sensitive to the reinforcing and locomotor stimulant effects of opioids and have greater opioid receptor density compared to males (Craft, 2008). Further elucidation of this underlying neurobiological basis will be crucial in expanding our understanding of the sex differences seen regarding opioids as well as kratom.

Current Study

The aim of the proposed study is to examine the effects of MG and 7-HMG on phasic dopamine release in the NAc. Stimulation-evoked dopamine release will be quantified using *in vivo* fixed potential amperometry in anesthetized mice before and after administration of MG, 7-HMG, or vehicle. Fixed potential amperometry, also known as continuous amperometry, coupled with carbon fiber recording microelectrodes is an established technique for real-time monitoring of stimulation-evoked dopamine release (Dugast et al., 1994; Suaud-Chagny et al., 1995; Lester et al., 2010; Holloway et al., 2018). Given the recurrence of dose-dependent effects in previous studies with these alkaloids, we will administer three doses of each alkaloid representing low, medium, and high doses. Administering MG or 7-HMG during phasic dopamine recordings will allow for an assessment of the impact of these alkaloids on three measurements related to dopamine transmission: dopamine release (magnitude of the dopamine response), dopamine synaptic half-life (the time dopamine remains in the synapse, an indication of DAT functioning), and dopamine autoreceptor sensitivity (an indication of presynaptic D2 functioning). We

hypothesize that both alkaloids, at all doses, will increase NAc dopamine release to some degree, with low doses producing greater dopaminergic effects compared to higher doses and 7-HMG producing greater dopaminergic effects than MG. Given the underlying neurobiological factors we also hypothesize that females will exhibit greater dopaminergic effects compared to males across both alkaloids and at all doses. This study will improve our understanding of the neurochemical mechanisms of kratom. Specifically, the proposed study will provide insight on the impact of kratom on mesolimbic dopamine functioning and, consequentially, the abuse liability of this drug.

Methods

Mice

Eighty-four C57BL/6J mice (42 male, 42 female) (Jackson Laboratories, Bar Harbor, ME) will be housed five per cage in a temperature-controlled environment (21 ± 1 °C) on a 12-h light/dark cycle (lights on at 0600). Food and water will be available *ad libitum*. All mice will be 12-20 weeks old at the time of experiments. All experiments were approved by the Institutional Animal Care and Use Committee at the University of Memphis will be conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. In order to maintain the principle of reduction related to scientific experiments on animals (Kilkenny et al., 2010), efforts will be made to reduce the number of mice used. Efforts will also made to minimize pain and discomfort.

Surgery and Dopamine Recordings

Mice will be anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame (David Kopf Instruments, Tujundga, CA) ensuring flat skull. Body temperature shall be maintained at 36 ± 0.5 °C with a temperature-regulated heating pad (TC-1000; CWE, NY). Following a longitudinal incision to expose the skull surface, three trephine holes (1 mm o.d.) will be drilled. All stereotaxic coordinates have been confirmed in previous studies from our lab (Lester et al., 2010; Holloway et al., 2018) and are presented in mm from bregma, midline, and dura according to the mouse atlas of Paxinos and Franklin (2001). A stimulating electrode (SNE-100; MicroProbes, Gaithersburg, MD) will be inserted into the left VTA (AP −3.3, ML +0.3, and DV −4.0). A carbon fiber recording electrode with an active recording surface of 500 μm (length) by 7 μm (o.d.) will be implanted in the NAc (AP + 1.5, ML +1.0, and DV −4.0) (Figure 1). A stainless-steel auxiliary and Ag/AgCl reference electrode combination will be positioned in contact with contralateral cortical tissue 2.0 mm posterior to bregma. All amperometric recordings will be made within a Faraday cage to increase signal to noise ratio. A fixed potential (+0.8 V) will be applied to the recording electrode, and oxidation current monitored continuously (10 K samples/s) with an electrometer (ED401 e-corder 401 and EA162 Picostat, eDAQ Inc., Colorado Springs, CO) filtered at 50 Hz (see Figure 1).
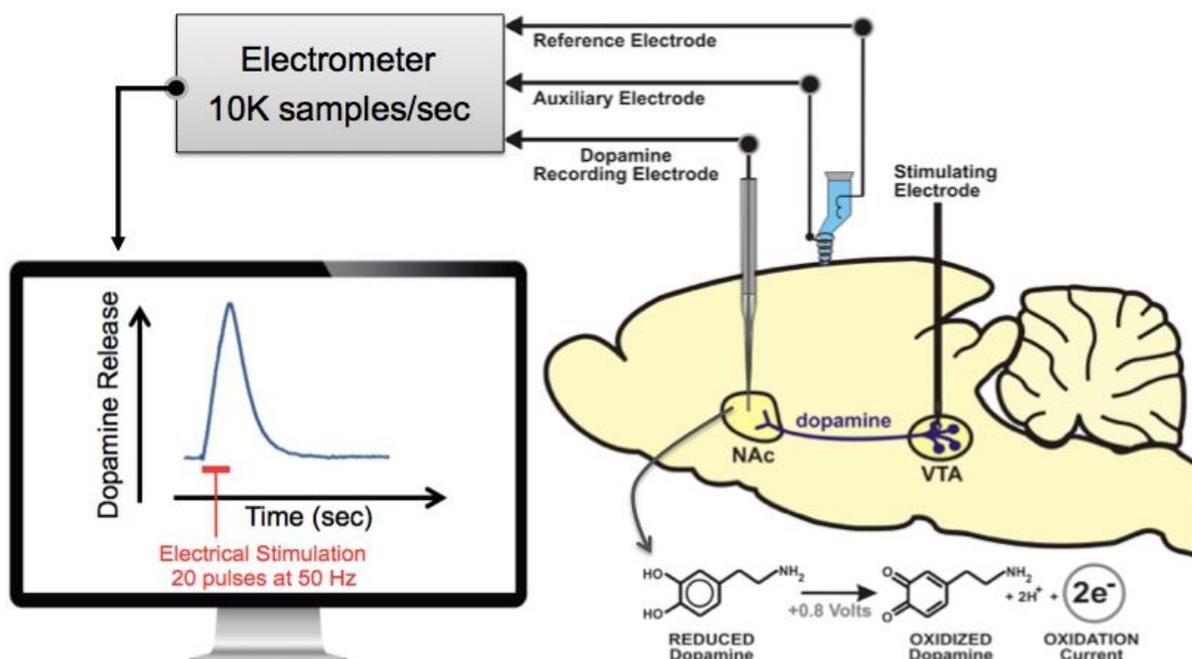
Figure 1. Amperometric Set-up. In vivo fixed potential amperometry will used to quantify nucleus accumbens (NAc) dopamine release evoked by stimulation of dopamine cell bodies within the ventral tegmental area (VTA).

A series of cathodal monophasic current pulses, 20 pulses 0.5 ms in duration (800 µAmps) at 50 Hz every 30 s, was delivered to the stimulating electrode by an optical isolator and programmable pulse generator (Iso-Flex/Master-8; AMPI, Jerusalem, Israel). Following a 10-min baseline recording of stimulation-evoked dopamine release, mice received an i.p. injection of either vehicle (20% Tween80 with saline), mitragynine (MG) (1, 15, or 30 mg/kg), or 7-Hydroxymitragynine (7-HMG) (0.5, 1, or 2 mg/kg). See Table 1 for a breakdown of the experimental groups. Baseline stimulation parameters and recordings were continued for 30-min post injection whereupon dopamine autoreceptor sensitivity was assessed by applying a pair of test stimuli (T1 and T2, each 10 pulses at 50 Hz separated by 10 s) to the MFB every 30 s. Five sets of conditioning pulses (1, 5, 10, 20, and 40; 0.5 ms pulse duration at 15 Hz) were delivered prior to T2 with 0.3 s between the end of the conditioning pulse train and T2 (Holloway et al.,

2018; Mittleman et al., 2011). Autoreceptor-mediated inhibition of evoked dopamine release was expressed in terms of change in the amplitude of T2 with respect to T1 for each set of conditioning pulses (i.e., higher autoreceptor sensitivity results in lower amplitude of T2 relative to T1). Upon completion of the autoreceptor sensitivity test, stimulation parameters were reset to 20 pulses at 50 Hz every 30 s for 1 hour.

Table 1.

Number of Mice per Experimental Group

Sex	Drug	Dose	N
Male	MG	1 mg/kg	6
		15 mg/kg	7
		30 mg/kg	7
	7-HMG	0.5 mg/kg	6
		1 mg/kg	6
		2 mg/kg	6
	Vehicle	-	7
		Total Males	**45**
Female	MG	1 mg/kg	6
		15 mg/kg	6
		30 mg/kg	6
	7-HMG	0.5 mg/kg	6
		1 mg/kg	5
		2 mg/kg	5
	Vehicle	-	6
		Total Females	**40**
		TOTAL MICE	**85**

Note. MG: mitrgynine, 7-HMG: 7-hydroxymitragynine, vehicle: 20% Tween80 with saline

Following each experiment, an iron deposit was created to mark the stimulating electrode site by sending direct anodic current (100 μA for 10 s) through the electrode. Mice were then euthanized with intracardial injection of urethane. Brains were removed and stored in a 30% sucrose/10% formalin solution with 0.1% potassium ferricyanide. Also, following each experiment, carbon fiber electrodes calibrated *in vitro* using dopamine solutions (0.2–1.2 μM) administered with a flow injection system (Michael and Wightman, 1999; Prater et al., 2018). Thus, change in dopamine oxidation current (Amp) was converted to dopamine concentration (M).

Drugs

Urethane and dopamine were obtained from Sigma-Aldrich Chemical (St Louis, MO). Urethane was dissolved in distilled water, and dopamine was dissolved in phosphorate-buffered saline (PBS pH 7.4). MG and 7-HMG were obtained via Dr. Christopher McCurdy at the University of Florida and were dissolved in 20% Tween80 and saline (Hemby et al., 2018; Vuppala et al., 2013).

Data Analysis

In order to quantify the recorded dopamine efflux, data points occurring at 0.25 sec pre- and 10 sec post-stimulation will be extracted at the time of interest. Starting 1 minute before the drug injection to 1.5 hours post injection, stimulation-evoked responses will be analyzed every 10 minutes. At these time points, we will quantify dopamine release (the magnitude of the response peak) and dopamine half-life (i.e. the time for 50% decrease from the maximum evoked increase to the prestimulus baseline level). Mixed factorial ANOVAs will be used to determine the effects of sex, drug, and dose (between-subjects factors) on dopamine release and half-life over the 90-minute recording period (within-subjects factor). A mixed factorial ANOVA will

also be used to determine the effects of sex, drug, and dose (between-subjects factors) on autoreceptor functioning across the pre-pulse conditions (within-subjects factor). One-way ANOVAs with Tukey's HSD post-hoc tests will be used when appropriate to examine significant group differences. For all analyses, alpha will be set at .05.

Results

Baseline Dopamine Release and Half-Life

Stimulation-evoked dopamine efflux was recorded before and after kratom alkaloid administration (MG or 7-HMG). Baseline dopamine release (µM) and dopamine synaptic half-life (sec) were assessed in each mouse prior to the drug challenge. Neither baseline dopamine release or half-life differed between males and females (release: $[F(1,79) = .532, p = .468]$; half-life: $[F(1,79) = 2.840, p = .096, \eta_p^2 = .035]$. Furthermore, neither baseline dopamine release or half-life differed between drug groups (vehicle, MG, or 7-HMG) (release: $[F(2,79) = .763, p = .469]$; half-life: $[F(2,79) = .040, p = .960]$. These findings confirm that the randomly assigned experimental groups contained no significant differences in dopamine functioning before drug administration (see Figure 3A-C).
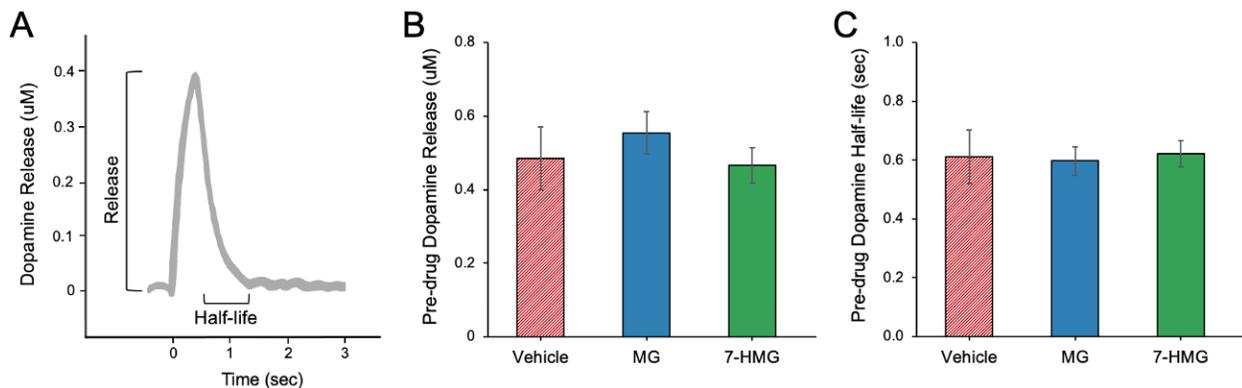
Figure 3. Baseline dopamine release and half-life. (A) Example response. No significant differences in (B) dopamine release or (C) half-life were observed between housing or drug groups. Data is displayed as mean ± SEM.

Dopaminergic Response to MG

A mixed factorial ANOVA was used to determine the effects of sex and dose (0, 1, 15, and 30 mg/kg MG) (between-subjects factors) on percent change in dopamine release throughout the 90-minute recording period (in 10-minute intervals, within-subjects factor). Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, $\chi^2(44) = 402.97$, $p < .001$, requiring the Greenhouse–Geisser correction be applied for evaluating main effects and interactions. There was a significant main effect of time post injection on percent change in dopamine release [$F(2.71,116.69) = 193.12$, $p < .001$, $\eta_p^2 = .818$]. There was a significant interaction between time and sex on percent change in dopamine release [$F(2.71, 116.69) = 2.83$, $p = .047$, $\eta_p^2 = .062$], but no significant interaction between time and dose [$F(8.14,116.69) = .740$, $p = .658$]. A significant three-way time x sex x dose interaction was observed [$F(8.14,116.69) = 2.25$, $p = .028$, $\eta_p^2 = .136$]; thus follow-up analyses at each time point were employed. For each sex, one-way ANOVAs at each time point revealed no significant release differences between doses ($p > .05$) (see Figure 4A -B). While not significant at any specific time point, our results indicate MG does have the ability to alter dopaminergic efflux over time depending on sex and dose administered.

Regarding percent change in dopamine half-life following MG, Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, $\chi^2(44) = 166.49$, p $< .001$, requiring the Greenhouse–Geisser correction be applied for evaluating main effects and

interactions. There was a significant main effect of time post MG injection [$F(3.62,155.62)$ = 2.53, p = .048, η_p^2 = .056], but no other interactions between time x sex [$F(3.62,155.62)$ = 1.36, p = .253], time x dose [$F(10.86,155.62)$ = .850, p = .589], or time x sex x dose [$F(10.86,155.62)$ = .541, p = .871] significantly altered dopamine half-life.

Dopaminergic Response to 7-HMG

A mixed factorial ANOVA was used to determine the effects of sex and dose (0, 0.5, 1, and 2 mg/kg 7-HMG) (between-subjects factors) on percent change of dopamine release throughout the 90-minute recording period (in 10-minute intervals, within-subjects factor). Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, $\chi^2(44)$ = 449.98, $p < .001$, requiring the Greenhouse–Geisser correction be applied for evaluating main effects and interactions. There was a significant main effect of time post injection on percent change in dopamine release [$F(2.39,93.34)$ = 117.49, $p < .001$, η_p^2 = .751], but no significant interaction between time and sex [$F(2.39,93.34)$ = .60 , p = .58], meaning there were no significant differences between male or female groups at any given time point. There was a significant interaction between time and dose on percent change in dopamine release (significance is dependent on specific time points and doses of 7-HMG) [$F(7.18,93.34)$ = 4.70, $p < .001$, η_p^2 = .266], but no significant time x sex x dose interaction [$F(7.18,93.34)$ = 1.24, p = .288]. Given no effects of sex, follow-up analyses on the 7-HMG dose effect on dopamine release were carried out with the male and female data combined. One-way ANOVAs at each time point revealed significant dose effects on dopamine release at several time points (40 min: p = .039; 60 min: p = .039; 70 min: p = .026; 80 min: p = .019; and 90 min: p = .026) (see Figure 3C). Tukey HSD post hoc tests further distinguished these group differences. Specifically, there

were significant differences in release between the vehicle and high dose 7-HMG at 40 min and between the low and high dose 7-HMG from 60-90 min ($p < .05$). (see Figure 4D-E).

Regarding percent change in dopamine half-life following 7-HMG, Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, $\chi2(44) = 176.64$, p $< .001$, requiring the Greenhouse–Geisser correction be applied for evaluating main effects and interactions. There was no significant main effect of time [$F(3.93,153.13) = 2.12$, $p = .083$, $\eta_p^2 = .052$], time x sex [$F(3.93,153.13) = .659$, $p = .619$], time x dose [$F(11.78,153.13) = .690$, $p = .756$], or time x sex x dose [$F(11.78,153.13) = 1.158$, $p = .319$].
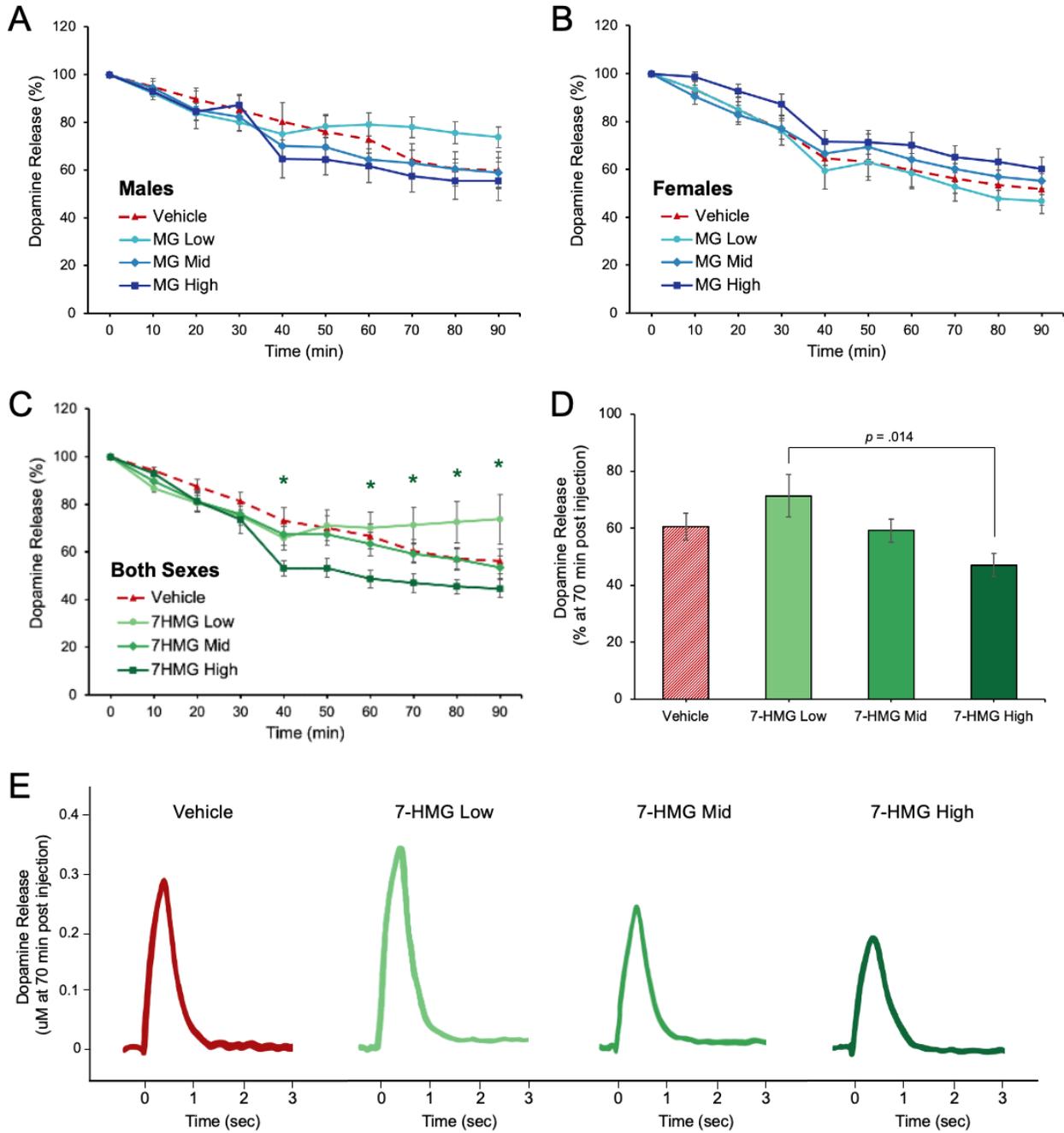
Figure 4. Dopamine release following drug challenge. (A) No significant differences in

dopamine release were observed for males or (B) females at any dose tested. (C) Significant

differences in release were observed between the vehicle and high dose 7-HMG at 40 min and

between the low and high dose 7-HMG from 60-90 min for both sexes. (D) Example of

significant increase in stimulated dopamine release at 70 minutes. (E) Representative responses

from each dose of 7-HMG and VEH at 70 minutes.

Dopamine Autoreceptor Functioning

For the assessment of autoreceptor functioning a mixed factorial ANOVA was used to

determine the potential effects of sex and drug dose (between-subjects factors) on autoreceptor

functioning across varying pre-pulse settings (within-subjects factor). Autoreceptor-mediated

inhibition of evoked dopamine release was expressed in terms of the percentage change between

test stimulations (T1 and T2) for each set of conditioning pulses (or pre-pulses). With 0 pre-pulse

stimulation pairs near 100% dopamine release (no change between T1 and T2), percent release

of T2/T1 that is decreased from 100% indicates autoreceptor-mediated inhibition of dopamine

release (see Figure 5A-B). Thus, greater decreases in dopamine release (T2/T1) indicates

increased autoreceptor functioning. Assessments of autoreceptor functioning were conducted 20-

minutes post-injection across all groups.

Regarding autoreceptor functioning following MG administration, Mauchly's test of

sphericity indicated that the assumption of sphericity had been violated, $\chi 2(20) = 177.19$, p

$< .001$, requiring the Greenhouse–Geisser correction be applied for evaluating main effects and

interactions. There was a significant main effect of number of pre-pulses on percent change in

autoreceptor-mediated dopamine release [$F(2.12,91.18) = 238.01$, $p < .001$, $\eta_p^2 = .847$],

validating the test by confirming that the number of pre-pulses impacted dopamine release from

T1 to T2. There were not significant interactions between pre-pulses x sex [$F(2.12,91.18) = .417$,

$p = .672$] or pre-pulses x drug dose [$F(6.36,91.18) = 1.94$ $p = .079$, $\eta_p^2 = .119$]. However, there

was a significant 3-way interaction between pre-pulses x sex x drug dose [$F(6.36,91) = 2.24$, p

= .043, η_p^2 = .135], indicating that MG altered autoreceptor functioning differently in males and females. In males, follow-up tests indicated significant differences in autoreceptor-mediated dopamine release between dose groups at 20 pre-pulses [$F(3,23) = 3.76$, $p = .025$, η_p^2 = .329] and 40 pre-pulses [$F(3,23) = 3.27$, $p = .039$, η_p^2 = .299](see Figure 5C), with the low dose MG mice displaying significantly increased autoreceptor functioning relative to vehicle mice ($p < .05$). In females, follow-up tests revealed no significant differences at any specific pre-pulse setting ($p > .05$) (see Figure 5D).

Regarding autoreceptor functioning following 7-HMG, Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, $\chi2(20) = 144.81$, $p < .001$, requiring the Greenhouse–Geisser correction be applied for evaluating main effects and interactions. There was a significant main effect of number of pre-pulses on autoreceptor-mediated dopamine release [$F(2.31,90.09) = 261.35$, $p < .001$, η_p^2 = .870], again indicating that the number of pre-pulses impacted autoreceptor-mediated dopamine inhibition. There were not significant interactions between pre-pulses and sex [$F(2.31,90.09) = .933$, $p = .408$], pre-pulses and drug dose [$F(6.9,90.09) = 1.32$ $p = .252$], or pre-pulses x sex x drug dose [$F(6.93,90.09) = .987$, $p = .446$]. No follow-up analyses were conducted as 7-HMG did not alter autoreceptor-mediated dopamine release (see Figure 4E).
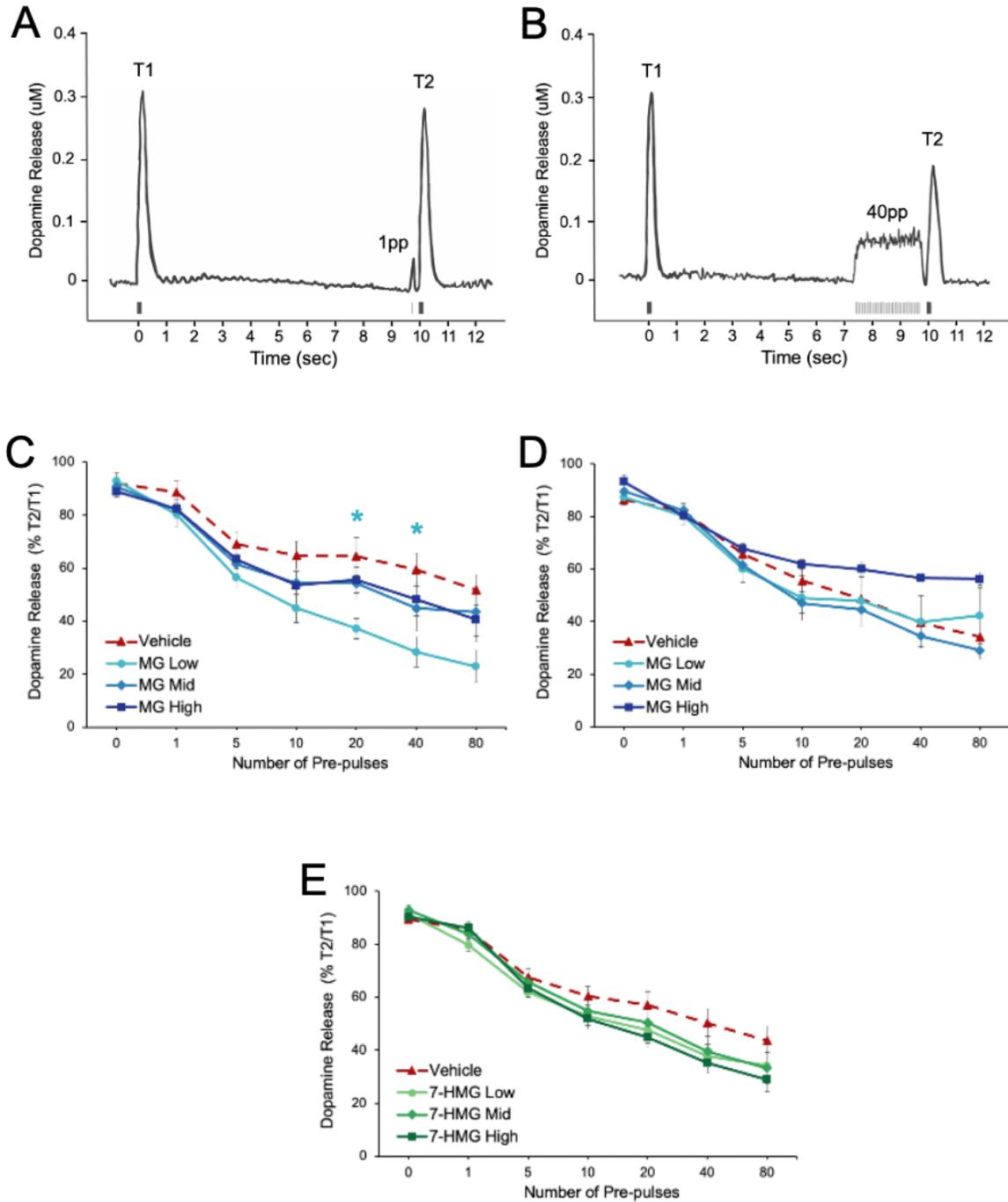
Figure 5. Autoreceptor-mediated inhibition of dopamine release. (A) Example amperometric recording of autoreceptor test stimulations (T1,T2) separated by 1 pre-pulse (pp) and (B) 40 pre-pulses. Greater decreases in dopamine release (% of T2/T1) indicates increased autoreceptor functioning**.** (C) Low dose MG significantly increased autoreceptor functioning (greater decrease

in dopamine release) in males relative to vehicle mice at 20 and 40 pre-pulses. (D) In females, no significant differences at any specific pre-pulse setting were observed. (E) 7-HMG did not alter autoreceptor-mediated dopamine release at any dose tested.

Discussion

The aim of the proposed study was to examine the effects of kratom's two primary alkaloids, MG and 7-HMG, on phasic dopamine release in the NAc, an area often targeted by drugs of abuse. *In vivo* fixed potential amperometry was used to quantify VTA stimulation-evoked dopamine release in anesthetized mice. During dopamine recordings, mice received an i.p. injection of either MG (1, 15, or 30 mg/kg i.p.), 7-HMG (0.5, 1, or 2 mg/kg i.p.), or vehicle, and dopamine recordings continued for 90 minutes. We examined the effects of these drugs on dopamine release concentrations, the synaptic half-life of dopamine, and dopamine autoreceptor functioning. Both MG and 7-HMG dose-dependently altered aspects of stimulation-evoked dopamine release, but in different ways, suggesting different mechanisms at play between the two kratom alkaloids.

Effects of MG on phasic dopamine release

The effects of MG on stimulation-evoked dopamine release were both sex- and dose-dependent. We observed a significant time x sex x MG dose interaction, indicating that the amount of dopamine released across the 90 min recording period was altered differently in males and females based on the dose of MG administered. As shown in Figure 3A, it appears that for males the low MG dose (1 mg/kg) lead to a response pattern separate from the other doses, in the direction of increased dopamine release. However, follow-up tests did not show significant

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differences in release at any of the specific time points in either males or females. More research

is needed to determine what this may mean. Perhaps examining more dosages in the lower dose

range (such as 0.5 or 2 mg/kg) would shed light on these findings. While no others have

examined the effect of MG on dopamine release, our findings coincide to some degree with what

is currently available in pre-clinical behavioral paradigms and clinical reports. In animal models

utilizing males, acute MG administration at low dose (1 mg/kg i.p.) has been found to induce

significant locomotor activity and rearing behaviors compared to the vehicle group (Yusoff et.

al., 2016; Hazim et al., 2011). Similarly, in regions such as Southeast Asia kratom leaves are

historically and commonly chewed predominantly by men throughout the workday who

reportedly utilize its complex stimulant effects in the morning to combat fatigue and improve

work productivity (Suwanlert, 1975; Ahmad et al. 2012). All of these findings suggest that the

low MG doses have neural actions similar to stimulants. Regarding the lack of response to MG

in females, females have been shown to have a smaller accumbal response to drug stimulants

compared to males, but then exhibit more rapid acquisition and escalation of drug taking

compared to males (see Becker, 2016). Thus future studies examining the effects of repeated MG

administration on dopamine release are needed.

Extracellular dopamine concentrations are mediated by a large number of neural

mechanisms, two of which are the functioning of DAT and dopamine autoreceptors. As

measured in the current study, the synaptic half-life of dopamine conveys the amount of time

required to clear dopamine from the synapse. Therefore, the synaptic half-life of dopamine is an

indication of DAT functioning, with an increased half-life indicating decreased DAT functioning

(Ford, 2014). MG administration did not alter the dopamine half-life over the 90 min recording

period, indicating that MG did not act on DAT. Many psychostimulants (such as cocaine and

amphetamine) inhibit DAT and dramatically increase the synaptic half-life of evoked dopamine (Lester et al., 2010; Holloway et al., 2018), but MG does not appear to share this mechanism with other psychostimulants. Yusoff et al. (2014) found that repeated administration of MG did not alter the number of DAT sites in the NAc but did increase the number of DAT sites in the mesencephalon (which houses the VTA). Thus, measurements of somatodendritic dopamine release in the VTA following MG may provide more information about the effects of MG on DAT in this pathway.

The high temporal resolution of fixed potential amperometry allows for the assessment of autoreceptor-mediated inhibition of dopamine release (Holloway et al., 2018; Mittleman et al., 2011). We observed a significant interaction between the number of pre-pulses applied, sex, and MG dose, indicating that the dose of MG administered altered the way male and female mice responded to the autoreceptor test. Specifically, the low dose of MG significantly increased dopamine autoreceptor functioning in males, leading to decreased dopamine release during these assessments. No significant effects of MG on autoreceptor functioning were observed in females. Previous research has established that dopamine autoreceptor sensitivity can be an indication of presynaptic D2 functioning (Ford, 2014) and mitragynine has been shown to have partial affinity for D2 dopamine receptors (Boyer et al., 2008). The present findings suggest that MG acts as a D2 agonist at low doses in males. Similarly, Stolt et al. (2014) found that kratom extract (at 2 mg/kg) decreased locomotor activity and that this effect was counteracted by a low dose of apomorphine, leading the authors to conclude that this dopaminergic effect of kratom was presynaptic (functionally dopamine antagonistic). Furthermore, the behavioral effects of MG have been compared to those of antipsychotics, which are typically dopamine antagonists (Johnson et al., 2020; Vijeepallam et al., 2016). Most antipsychotics are D2 receptor antagonists

(at post-synaptic sites), which has led some researchers to suggest that MG may antagonize D2 receptors (Johnson et al., 2020; Vijeepallam et al., 2016). Some antipsychotics have a dual mode of action on D2 receptors, acting on presynaptic sites at low doses and postsynaptic sites at high doses (Müller-Spahn, 2002). It is possible that MG has a similar dose-dependent effect on D2 sites (Stolt et al., 2014). The technique used in the present study would not detect actions at post-synaptic D2 receptor sites; thus, more research is needed to elucidate these dose- and sex-dependent effects of MG on D2 functioning.

Effects of 7-HMG on phasic dopamine release

The effects of 7-HMG on the measured aspects of phasic dopamine release were different than that of MG, suggesting the use of alternate neural mechanisms. Unlike MG, the dopaminergic effects of 7-HMG were not sex-dependent; however, similar to MG, the dopaminergic effects of 7-HMG were dose-dependent. We observed a significant time x 7-HMG dose interaction on dopamine release, indicating that the amount of dopamine released across the 90 min recording period was altered by 7-HMG. Specifically, 7-HMG at the lowest dose tested (0.5 mg/kg) significantly increased dopamine release from 60-90 minutes post injection, although not to the extent of known drugs of abuse. Interestingly, the highest dose of 7-HMG decreased dopamine release and whether this decrease is comparable to other therapeutic drugs remains to be seen. Although not conclusive, this notable dose-dependent release is currently leading researchers to compare kratom's D2 binding effects to medications that treat the positive symptoms of psychosis such as haloperidol and chlorpromazine (Vijeepallam, Pandy, Kunasegaran, Murugan, and Naidu, 2016). Additionally, even though we did not see any significant differences regarding 7-HMG and half-life, emerging research has also suggested that

7-HMG may have a limited capability to cross the blood-brain barrier and longer elimination half-life (2.4x) compared to MG (Maxwell et al., 2021).

Albeit these results show MG and 7-HMG do seem to have the potential to alter dopamine release in a dose dependent manner. There is also emerging evidence that indicates kratom's alkaloids activate α-2 adrenergic postsynaptic receptors which are present in modulatory pain pathways (Eastlack, 2020), without the recruitment of the beta-arrestin 2 pathway which is thought to be responsible for the undesirable effects seen in traditional opioids such as respiratory depression, constipation, and dependence (Johnson et al., 2020). Furthermore, when compared to the risk of evoked respiratory depression and overdose rates associated with high doses of traditional prescription opioids (mu-opioid receptor agonists), additional stringent investigation into kratom's unique pharmacology could offer drug development researchers a compound with comparable analgesic, anxiolytic, or antidepressant effects with a better safety profile (ex: exclusively oral route of administration), therapeutic index, or lower abuse liability. Some researchers even estimate the risk of overdose death is >1000 times greater for opioids than for kratom (Henningfield, 2019). Particularly when considering preliminary data from the CDC (2021) indicating drug overdose deaths in the United States rose 29.4% in 2020 to 93,331 (69,710 involving opioids), the investigation of novel treatments has become critical in addressing our ever-growing crisis. However, while our results can provide valuable insight regarding kratom's potential abuse liability and/or therapeutic utility (from previously mentioned studies), additional investigations will inevitably be paramount in furthering our understanding of this largely obscure plant and its novel alkaloids.

Indications of abuse liability

Our findings suggested the increases in dopamine release were considerably less than those normally observed in stimulants with a high abuse liability. More precisely in males, our results indicated low dose MG (1 mg/kg) increased dopamine release over time (+23% from vehicle for 70-90mins), but also increased dopamine autoreceptor functioning (+50% from vehicle). Comparatively, other studies from our lab have shown increases (329% ± 45) (322.53% ± 60.48) in intra-NAC dopamine release from psychostimulant administration (Holloway et al., 2018; Freels et al., 2020). Previous research has also shown low dose MG (1mg/kg i.p.) induced profound hyperlocomotion and rearing behavior compared with vehicle group (Yusoff et. al., 2016; Hazim et al., 2011). Pertaining to the significance of our findings regarding increased dopamine autoreceptor functioning (decreased release) at low dose MG (1 mg/kg), prior findings in rats have revealed MG exposure at a low dose (25 μg/inf) significantly reduced morphine self-administration up to 3 days following the last exposure (Hemby et al., 2018) and MG pretreatment (0.1 to 3.0 mg/kg) reduced response rates maintained by heroin (Yue, Kopajtic, & Katz, 2018). Concerning the significant differences seen across male and female mice in dopamine release from 60-90 minutes, the low dose of 7-HMG (0.5 mg/kg) increased dopamine release (+18% from vehicle) whereas the high dose (2 mg/kg) decreased dopamine release (-22% from vehicle). However, similarly to MG, our observed increase in dopamine release at low dose 7-HMG (+18%) was considerably less than those seen in the NAc following high dose of methadone (10 mg/kg) (+300%) or fentanyl infusions of 0.5 nmol (+132%), 2.5 nmol (+216%), and 5.0 nmol (+682%) (Di Chiara and Imperato, 1988; Yoshida et al., 1999). Lastly, despite the fact an increase in dopamine release is typically associated with a higher risk of abuse and greater likelihood of negative outcomes, the reduction of dopamine release at the highest 7-HMG

dose (2 mg/kg) tested is not without risk. The reduction in dopamine following high dose 7-HMG administration could potentially lead to a diminished interest in pleasurable activities or a reduction in the ability to experience pleasure itself, otherwise known as anhedonia (APA, 2013). Along with the potential loss of pleasure, anhedonia is oftentimes synonymous with depression and is associated with sedation, reduced motivation, and a reduced willingness to expend cognitive and physical effort for rewards (Horne, Topp, and Quigley, 2021). Furthermore, there is emerging evidence that opioid use disorder (OUD) has the greatest odds of being accompanied by anhedonia relative to other substance use disorders (SUD), although anhedonia is more likely to be seen in mood disorders and post-traumatic stress disorder (PTSD) (Stull et al., 2021). However, people with a history of PTSD, mood disorder, or OUD may be populations that are particularly vulnerable to anhedonia when seeking treatment for substance use disorders (Stull et al., 2021).

Limitations

The quantity and diversity of the alkaloids found in kratom, and its associated products, represent one of the most unique, pharmacologically complex substances that we are currently aware of. Kratom's relatively unknown status and ability to produce opiate-like and stimulant-like effects in a dose-dependent manner along with suspected mental health effects not only makes it an excellent candidate for drug development programs or potentially for treatment facilities, but also for labs investigating its abuse liability, mechanisms of action, or safety. While MG and 7-HMG are thought to represent the 2 major psychoactive alkaloids found in kratom, there are more than 3 dozen alkaloids found in kratom (Johnson et al., 2020), most of which there is scant research available. Unfortunately, one of the limitations within the realm of

preclinical evidence is that much of the research we currently have has been conducted using extracted MG and/or 7-HMG (not commercial products) via routes of administration that are not typically seen in human populations. Additionally, future studies could explore variations in housing, age, enrichment, pretreatment, or drug exposure time (acute vs chronic) prior to surgeries and dopamine recordings. Further pre-clinical investigations could also track the estrus cycles of their female rodents to better illuminate any significant sex differences seen. It is also worth considering that in our study we are measuring a programable stimulated dopamine release on fixed schedules that may not be entirely consistent with the firing rates that drive reward outside of a laboratory setting or in combination with other substances or drugs of abuse. Lastly, and perhaps most importantly, further investigation into the combinations and variable concentrations of these alkaloids will be paramount in the exploration of kratom's neuropharmacological mechanisms and effects.

Conclusions

The present study aimed to determine the effect of the 2 main alkaloids in kratom (MG and 7-HMG) on reward-related neurotransmission using in vivo fixed potential amperometry to quantify stimulation-evoked dopamine release in the NAc of anesthetized male and female mice. In males, dopamine release over the recording period was alter based on MG dose, with the lowest dose (1 mg/kg) appearing to increase dopamine release. The low dose of MG also increased dopamine autoreceptor functioning in males. Neither of these MG responses were observed in females. No sex effects were observed following 7-HMG. Dopamine release over the recording period was altered by the dose of 7-HMG, with dopamine release being significantly greater following the low dose compared to the high dose (with the control mice

responding in the middle). By providing insight on some of the neurochemical effect of components within kratom, a relatively new and readily available opioid, these results will join a growing body of research that will inevitably help provide vital information surrounding one of the United States' largest public health issues, the opioid crisis. The understanding of this greater context in which kratom is nestled and having the necessary information regarding its abuse potential, mortality risk associated with the combination of other substances/medications, or therapeutic utility is imperative in the current epidemic of opioid abuse. Our results show that kratom does have the potential to dose-dependently alter dopamine release, but not to the extent of traditional drugs of abuse (such as cocaine or morphine). Historically, kratom has exclusively been associated with opioids or stimulants, but recently its alkaloids have also begun to be considered for their unique targeting of mu opioid, serotonin, and dopamine receptors as innovative treatments for pain relief, opioid withdrawal, and various mental health disorders. Ultimately, this study has increased our understanding of kratom's 2 major alkaloids and their abilities to alter dopamine release, but further investigation surrounding the neural mechanisms of kratom's alkaloids and their interactions will provide crucial and urgent insight into their therapeutic uses or potential abuse liability.

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