1-1-2013

Evaluating preference-seeking and aversive qualities of Salvia divinorumand Mitragyna speciosa

Melissa Jane Loria
University of Mississippi

EVALUATING PREFERENCE-SEEKING AND AVERSIVE QUALITIES OF *SALVIA DIVINORUM* AND *MITRAGYNA SPECIOSA*

A Thesis
presented in partial fulfillment of the requirements
for the degree of Master of Arts
in the Department of Psychology
The University of Mississippi

by

MELISSA JANE LORIA

May 2013

ABSTRACT

Use of botanicals for self-diagnosed conditions or recreational purposes is increasing. Many readily available botanicals are under-researched and little is known about potential liabilities. This study sought to utilize Conditioned Place Preference (CPP) to quantify rewarding or aversive properties of botanicals and their constituents in rats. This paradigm is based on the notion that animals prefer distinct environments previously paired with rewarding drugs. Our approach to broadly characterize a botanical and its many constituents entails dose response functions of a plant extract, its fractions, and its primary constituent. As a proof of concept, we chose two popular botanicals, one expected to produce place preference (*Mitragyna speciosa*) and the other expected to produce place aversion (*Salvia divinorum*). Because salvinorin A is well characterized as the major psychoactive compound, we did not study an extract fraction for *S. divinorum*. Following apparatus habituation and quantification of baseline compartment preference, male Sprague-Dawley rats were given eight drug-compartment conditioning trials which involved alternate day pairings of test compound to one compartment and vehicle to the other. This was followed by a compartment preference trial conducted under drug-free states. As expected, rats showed place preference to mitragynine similar to that of 1mg/kg (+)-Amphetamine. This effect was much less pronounced with the extract and its fractions. Rats showed robust place aversion to both *Salvia divinorum* and salvinorin A. These findings suggest that products that contain significant quantities of mitragynine pose an abuse liability.

DEDICATION

For my Mom and Dad, who pushed me,

For Megan, who saw me through.

And for Brent, Michael, and Solomon, who kept me smiling.

ACKNOWLEDGEMENTS

To my committee members—Drs. Kenneth Sufka, Ikhlas Khan, and John Young—, thank you for your support, feedback, and time. I will be forever appreciative of your careful attention and endless support.

Special thanks to my advisor, Dr. Sufka. You have been such a wonderful guide and have created an atmosphere in which I can challenge myself but also find a sense of brotherhood and togetherness.

Lastly, a big thank you to all the lab members — undergraduate assistants, graduate students, and those just dedicating their time. Your constant attention and care for these studies were what made this possible.

TABLE OF CONTENTS

INTRODUCTION

Botanicals are entering the consumer market at a rapid pace and are widely available to consumers in a variety of forms. These products are often purchased by consumers to treat medical disturbances as well as for recreational purposes (Dennehy, Tsourounis, & Miller, 2005). In many cases, these plants are on the market with little knowledge of their range of action, toxicity, and abuse potential. However, consumers are comforted by the "all natural" label and continue to use the products because they falsely believe that this label indicates safety and a lack of physical and psychological health risks (Marcus & Grollman, 2002). Botanicals can be dangerously complex due to their wide arrays of constituents. While one effect of a botanical may be understood, numerous other constituents present in the same plant may lead to harmful effects. Even if the activity of the primary active constituent is understood, this knowledge may not be enough to have a full understanding of the plant's potential toxicity risks. Wide availability of under-researched botanical compounds could easily lead to a plethora of physiological and psychological disturbances in an unsuspecting group of consumers.

Kava-kava is an example of a botanical that found its way into the consumer market and led to severe toxicity in some users. The compound is extracted from the roots of *Piper mesthysticum*, a kava plant native to the South Pacific Oceanic Islands. Islanders traditionally consumed the drink for its intoxicating effects. Expansive knowledge on traditional use led to no worrisome side effects or glaring health risks (Whitton et al., 2003).

Kava-kava gained popularity in the West and modern consumers used it to aid in sleep, reduce anxiety, and as an alternative intoxicant to alcohol (Whitton et al., 2003). The Drug Enforcement Administration's Office of Diversion Control states on its Drug and Chemicals of Concern list that kava is sold as a dietary supplement and serves as a natural alternative to anti-anxiety medications. They also state that, while research on illicit use of kava is purely anecdotal, consumers are utilizing the plant for relaxation and its mild euphoric states. In addition, it is often used as a sleep aid ("Drugs and Chemicals of Concern: Kava").

The increased popularity of kava-kava was not without adverse consequences. Hepatotoxicity has been reported in a number of individuals regularly using kava (Humberston, Akhtar, & Krenzelok, 2003; Teschke, Schwarzenboeck, & Akinci, 2008). More specifically, liver damage, including hepatitis and cirrhosis, and full liver failure have been linked to kava use ("Drugs and Chemicals of Concern: Kava").

Observations of toxicity associated with kava-kava are not surprising given current analysis of this product. In an *in vitro* model of liver toxicity, kava extracts were found to cause toxic effects (Lüde et al., 2008). Further, kavalactones were shown to disrupt liver detoxification pathways due to inhibition of cytochrome P450 activity in addition to reducing the natural levels of glutathione in the liver (Clouatre, 2004).

Present-day consumers are engaged in self-diagnosing and using wholly unregulated botanicals for the treatment of a variety of maladies. They are being prescribed and self-prescribing unstandardized botanicals without any empirical evidence for efficacy or any empirical knowledge of potential toxicity. Further, no systematic studies have evaluated proper dosing and possible drug-drug interactions in many of these compounds. These issues alone would justify the need of a scientific approach for systematically studying these botanicals.

The recreational use of botanicals marketed to produce pleasurable psychoactive effects is another major concern. These botanicals are widely available at local "head shops" and through online botanical pharmacies (Dennehy, Tsourounis, & Miller, 2005). One attraction to the use of understudied botanicals as recreational compounds is that they are unregulated. As with botanicals used medicinally, recreational botanicals have not been properly evaluated for abuse liability or for toxicity associated with the product.

Animal models are often used to evaluate properties of drug substances. A common animal model used to identify abuse potential is the Conditioned Place Preference paradigm (CPP). CPP is based on the notion that animals prefer environments previously paired with positively reinforcing drugs (Bardo & Bevins, 2000). The paradigm has three phases: 1) habituation and initial compartment preference, 2) conditioning through pairing drug/no-drug treatments in chambers with distinct stimuli, and 3) testing the conditioned animal under drug-free states. Preference is determined by animals spending significantly more time in the drug-paired chamber and aversion is determined by animals spending significantly less time in the drug-paired chamber.

Because botanical products typically contain a large array of constituents, determining abuse potential is more challenging. This is due to the possibility of the plant containing more than one psychoactive constituent with rewarding or antagonistic effects. For example, a botanical may have a primary active constituent with abuse potential, but in combination with antagonistic constituents present, it will not indicate preference. Thus, studying entire plants for abuse potential could lead to false negatives in this paradigm. On the other hand, studying individual constituents could also lead to false negatives. This could occur if preference relies on the synergistic effects of multiple constituents.

A solution to the previously outlined problem is a methodical approach in which the whole plant extract, a fraction of the extract, and the plant's believed active constituent are evaluated. With this approach, the paradigm can identify a) if the believed active constituent is wholly responsible for a plant's psychoactive effects, b) if there are further rewarding or aversive qualities in other constituents, and c) if the constituents responsible for alternative qualities are present in the selected fraction. The process of identifying other psychoactive constituents can be narrowed down by evaluating whether the fraction shows the same qualities of the whole plant extract. If so, the fraction will be known to contain the responsible constituent. If not, further evaluation of constituents present in the whole plant extract will be necessary. In either case, it will be highlighted that there are additional psychoactive properties in the plant.

As a proof of concept, two products were evaluated using this method: one expected to produce place preference and another expected to produce place aversion. These expectations were developed based on human responses to recreational or medicinal use, broad historical backgrounds and traditional uses, and an understanding of receptor binding activity in the two plants. Of the many available, we selected *Salvia divinorum* (*S. divinorum)* to serve as the aversive botanical and *Mitragyna speciosa* (*M. speciosa*) to serve as the rewarding botanical.

S. divinorum, a member of the Sage family and native to Mexico, is also known as "Diviner's Sage," "Mystic Sage," and "Magic Mint" (Babu, McCurdy, & Boyer, 2008). *S. divinorum* was traditionally consumed by means of water infusion (Valdéz III, Díaz, & Paul, 1983). The Drug Enforcement Administration has listed *S. divinorum* and its active constituent, salvinorin A, on the drug or chemical of concern list. Several states have already placed regulatory control on both *S. divinorum* and salvinorin A ("Drugs and Chemicals of Concern: *Salvia Divinorum*,"). Traditionally, the Mazatec tribe of Oaxaca, Mexico used *S. divinorum* for

both the divination of shamans through hallucinations as well as for curing a variety of physiological and psychological ailments in community members (Valdéz III et al., 1983). In the 1960s, nearly all Mazatec tribe members claimed to be aware of the botanical (Wasson, 1962).

Today, *S. divinorum* is in the spotlight because it is marketed as a legal hallucinogen and is readily available to consumers (Griffin, Miller, & Khey, 2008; Hillebrand, Olszewski, & Sedefov, 2010). Use of the botanical is growing rapidly in the young adult population with 5.9% of high schools seniors reporting to have used *S. divinorum* in the last year ("Drugs and Chemicals of Concern: *Salvia Divinorum*,"). In states without restrictions, *S. divinorum* can be purchased at "head shops." In addition, it is one of the most widely available herbal dietary supplements suggested for recreational use on the internet (Dennehy et al., 2005). Consumers are now using the plant by means of smoking the leaves or smoking pure salvinorin A ("Drugs and Chemicals of Concern: *Salvia Divinorum*,").

Salvinorin A is the known active alkaloid and other constituents in the plant are not believed to have psychoactive properties ("Drugs and Chemicals of Concern: *Salvia Divinorum*,"). Receptor binding studies have demonstrated that salvinorin A acts as a κ-opioid receptor agonist (Chavkin et al., 2004). Previous studies have shown that κ-agonists act as a psychotomimetic in humans and, not surprisingly, cause place aversion in rodent models (Mucha & Herz, 1985; Pfeiffer et al., 1986). Consistent with these findings, salvinorin A has been shown to produce place aversion in mice (Zhang et al., 2005).

M. speciosa, a member of the Rubiaceae family and native to Southeastern Asia, is widely known as "Kratom," but is also called "Biak-biak" and "Ketum" in Malaysia (Ahmad & Aziz, 2012). Consumption of the plant is primarily achieved through chewing fresh leaves but some users grind and swallow leaves or use the leaves in a tea (Assanangkornchai et al., 2007).

The Drug Enforcement Administration has listed mitragynine as the primary active alkaloid in *M. speciosa.* The plant has long been a part of southern Thailand history and traditions with its use first recorded in 1935. The botanical was medicinally used for pain, infection, coughing, and diarrhea (for review: Hassan et al., 2013).

A recent survey in northern Thailand revealed that most local *M. speciosa* users were consuming the plant to increase physical endurance for long, physically demanding work days (Ahmad & Aziz, 2012). Currently, western consumers have begun to purchase the botanical for its stimulant like effects at low doses and its opiate-like and sedative effects at higher doses ("Drugs and Chemicals of Concern: *Mitragyna speciosa*,"). These recreational uses of the plant are not isolated to the West; use amongst young people in Thailand is increasing due to the introduction of a homemade cocktail called 4x100. The cocktail includes boiled *M. speciosa* leaves, cough syrup, and Coca-Cola (Tanguay, 2011).

Mitragynine is the major active chemical constituent found in *M. speciosa* and is not found in any other *Mitragyna* plants (Shellard, 1974). Mitragynine has been shown to have high affinity to the μ-opioid receptor (Watanabe et al., 1997; Yamamoto et al., 1999). Previous studies have shown that μ-opioid receptor agonists play a role in addiction (for review Koob, Sanna, & Bloom, 1998). A study looking at current users revealed that 88% of daily users were unable to stop using the plant (Ahmad & Aziz, 2012). Not surprisingly, μ-opioid receptor agonists have been shown to produce place preference in a rodent model (Bardo, Rowlett, & Harris, 1995).

In the present project, rewarding and aversive properties of *S. divinorum* and *M. speciosa* will be characterized using the CPP model. The full plant extract and the major psychoactive constituent will be evaluated in both plants in addition to a fraction of the constituents in *M. speciosa.* Salvinorin A's CNS action and behavioral effects are well understood, but there is a

chance that other minor constituents possess antagonistic or synergistic effects. In testing the whole plant extract, the presence of any unknown psychoactive components or potential synergistic effects will be realized. Thus, to reduce animal use, a fraction of *S. divinorum* will not be evaluated.

METHODS

Male Sprague Dawley rats (175-200 g, 6-7 weeks old; Harlan, Indianapolis, IN) served as subjects for these experiments. Animals were housed in pairs and maintained under a 12-h light/dark cycle in a temperature and humidity controlled vivarium. Food and water were available *ad libitum*. Animals had a 3-day period in which they were not tested but handled by experimenters to acclimate to the vivarium and to reduce experimenter-related stress. In both experiments, conditions had a sample size of 7-10.

Five place preference chambers (Model MED CPP RS; Med Associates, St. Albans, VT) were used for the experiment. Each chamber has three compartments: two stimulus-distinct, drug-conditioning chambers and a centralized "neutral" start chamber. Guillotine doors provide access between the start chamber and the conditioning chambers. Each conditioning chamber measures 30 x 30 x 30 cm. The stimuli of the two conditioning compartments are distinct on the basis of visual and tactile cues. One compartment is white with wire mesh floor and the other compartment is black with a metal rod floor. The center chamber measures 15 x 30 x 30 cm and is gray with a solid floor.

The Conditioned Place Preference procedure (Bardo & Bevins, 2000) involves four phases: 1) a 15 minute apparatus habituation trial under drug free states, 2) a 15 minute baseline preference trial under drug free states, 3) eight 30 minute drug/drug-free conditioning trials, and 4) a final 15 minute place preference trial under drug-free states. Animals had access to the entire place preference apparatus during the habituation, baseline, and final preference trials. Baseline

preference trials were used to identify any initial compartment bias to aid in assigning compound pairing conditions. The conditioning phase was characterized by alternate day pairings of test compound in one compartment (S+ compartment) and vehicle in the other (S- compartment). S+ was determined by selecting the non-preferred side if the compound was expected to produce CPP and the preferred side for compounds expected to produce CPA. Test apparatus was thoroughly cleaned after each trial. Compound or vehicle was administered via intraperitoneal (IP) injection immediately preceding each conditioning trial. Conditioning trials were counter-balanced for order (drug/vehicle) within treatments conditions. Compartment and compound/vehicle assignments were also counter-balanced and assigned based on initial preference scores. Following the final phase of the procedure, the change in time spent in the S+ compartment pre- and post-conditioning was analyzed.

Drug Probes

Experiment I

Morphine has previously been used as a compound to establish preference seeking behavior in this paradigm (for review: Tzschentke, 2007). In this experiment, 10 mg/kg of Morphine served as a "positive" control for quantifying conditioned place preference (CPP). Haloperidol, on the other hand, has previously been used as a compound to establish aversion in this paradigm (for review: Tzschetke, 1998). 1 mg/kg of Haloperidol served as the "negative" control for quantifying conditioned place aversion (CPA). Morphine was diluted in physiological saline. 50% DMSO and 50% saline were used as the vehicle for haloperidol (1.0 ml/kg).

Salvia divinorum extract (10, 30, 100 mg/kg) was prepared by exhaustive extraction of dry plant material with ethanol. The extract was filtered and then concentrated. Because it is

well-established that the psychoactive properties of S. divinorum are mediated by salvinorin A, we opted to not include analysis of a S. divinorum fraction. Salvinorin A (0.1, 0.3, 1.0 mg/kg) was isolated from S. divinorum leaves as previously described (Munro & Rizzacasa, 2003). Briefly, S. divinorum leaves were extracted with acetone and subsequently recrystallized from 95% ethanol to yield 99% (HPLC) pure salvinorin A. The salvinorin A doses selected were based on previously published studies in rodent models (McCurdy et al, 2006) and, for S. divinorum, dosing equivalence based on concentrations of salvinorin A in the extract. 10% DMSO and 10% Tween80 were used as the vehicle for *S. divinorum* and salvinorin A (1.0 ml/kg).

Experiment II

In the second experiment, d-amphetamine (1 mg/kg), instead of morphine, served as the "positive" control. D-amphetamine has previously been used to establish preference seeking behavior in this paradigm (for review: Tzschetke, 1998). This was used because it was anticipated that *M. speciosa* may show some psychostimulant properties. Haloperidol (0.8 mg/kg) served as the "negative" control. Amphetamine was diluted in physiological saline. 50% DMSO and 50% saline were used as the vehicle for haloperidol (1.0 ml/kg).

The leaves of M. speciosa (Voucher # 12433) were exhaustively extracted with methanol and the solvent was removed under reduced pressure to yield a dried extract. An aliquot was suspended in 5% HCl in water and extracted with ethyl acetate. The water-soluble part was basified (pH 9-10) with liquid ammonia and extracted with ethyl acetate. The ethyl acetate-soluble part, separated from basic media, was dried under reduced pressure to get an alkaloid enrich fraction. Mitragynine (97% pure) was isolated from the alkaloid enrich fraction by

repeated column chromatogaraphy over silica gel using chloroform/methanol (9:1) and hexanes/acetone/liq. ammonia (210:90:1) solvent systems. From these processes, M. speciosa extract (750, 1500, 4500 mg/kg), alkaloid enrich fraction (187.5, 375, 1125 mg/kg) and mitragynine (75, 150, and 450 mg/kg) were used in this study. The mitragynine doses selected were based on previously published studies in rodent models (Sabetghadam et al, 2013) and for M. speciosa, the dosing equivalence based on concentrations of mitragynine in the extract. In this study, S(+)-amphetamine (1 mg/kg in saline) served as the positive control for CPP and haloperidol (0.8 mg/kg) served as the negative control for CPA. Two vehicles were used and tested in this study. 20% Tween80 and saline were used as the vehicle for *M. speciosa*, the fraction, and mitragynine.

Data Acquistion and Analyses

All data acquisition was handled by infrared photo-beam detection via MED-PC® IV and analyses were conducted using SPSS statistical software. Group differences (preference/aversion scores) were analyzed using one-way ANOVAs. Preference scores are defined as (time spent in S+ post-conditioning – time spend in S+ pre-conditioning). A small change in relative time between S+ and S- chambers is expected in the vehicle groups simply due to repeated exposures. Relevant post hoc analyses will be performed using Fisher's LSD. Rewarding or aversive properties of compounds were determined by a statistically significant ($p < 0.05$) increase or decrease in preference scores relative to vehicle treated rats.

RESULTS

Experiment 1

To reduce the number of animals used, 5 vehicle animals received the haloperidol vehicle and 5 received the *S. divinorum* vehicle. A one-way ANOVA comparing preference scores was conducted and revealed no significant differences between these two groups. Therefore, vehicle groups were combined prior to computation of the analyses detailed below.

Preference scores for Experiment I are summarized in Figure 1. As expected, vehicle group preference scores changed minimally, increasing only +23 seconds. This minor change score is now depicted as the x-axis in Figure 1. Morphine treated animals tended to have higher preference scores and haloperidol treated animals tended to have lower preference scores compared to vehicle treated groups. In general, both *S. divinorum* and salvinorin A show a pattern of place aversion across all doses tested. To illustrate the place preference/aversion effects across treatment groups, a one-way ANOVA was performed on these data and revealed a significant main effect for treatment condition, $F(8,73) = 3.986$, $p = 0.001$. Post-hoc analyses revealed that mean preference scores for the morphine (positive control) condition did not significantly differ from the vehicle. The mean preference score for haloperidol was not, but approached being, significantly lower than the vehicle group, ($p = 0.083$). Mean preference scores for 10 and 100 mg/kg *S. divinorum* were statistically significantly lower than the vehicle, ($p = 0.022$ and $p < 0.001$). The 30 mg/kg *S. divinorum* extract condition was not, but approached being, significantly lower than the vehicle group, ($p = 0.073$). The mean preference scores of the 0.3 and 1.0 mg/kg salvinorin A groups were statistically significantly lower than the vehicle

group, ($p = 0.004$ and $p = 0.004$). The mean preference score of the 0.1 mg/kg salvinorin A group was in the direction of being significantly lower than the vehicle, ($p = 0.051$).

Experiment 2

To reduce the number of animals used, 5 vehicle animals received the haloperidol vehicle and 5 received the *M. speciosa* vehicle. A one-way ANOVA comparing preference scores was conducted and revealed no significant differences between these two groups. Therefore, vehicle groups were combined prior to computation of the analyses detailed below.

Preference scores for Experiment II are summarized in Figure 2. As expected, vehicle group preference scores changed minimally, increasing approximately +30 seconds. This minor change score is now depicted as the x-axis in Figure 2. D-Amphetamine treated animals have higher preference scores and haloperidol treated animals tended to have lower preference scores. In general, the *M. speciosa* extract, its fraction, and its active, mitragynine, show a pattern of place preference across nearly all doses. To illustrate the place preference/aversion effects across treatment groups, a one-way ANOVA was performed on these data and revealed a significant main effect for treatment condition, $F(11,101) = 2.97$, $p = 0.002$. Post-hoc analyses revealed that the mean preference score for d-Amphetamine (positive control) condition significantly differed from the vehicle group, ($p = 0.016$). The mean preference score of haloperidol was in the direction of being significantly lower than the vehicle, ($p = 0.09$). Mean preference scores for 75 and 450 mg/kg mitragynine groups were significantly higher than the vehicle, ($p = 0.027$ and $p = 0.026$). Mean preference score for 150 mg/kg mitragynine group was not, but approached being, significantly higher than the vehicle group, ($p = 0.076$).

DISCUSSION

Experiment 1

The goal of the first experiment was to characterize rewarding or aversive properties of *S. divinorum* and salvinorin A. Following apparatus habituation and quantification of baseline compartment preference, male Sprague-Dawley rats were given eight drug-compartment conditioning trials, which involved alternate day pairings of test compound to one compartment (S+) and vehicle to the other (S-). This was followed by a compartment preference trial conducted under drug-free states. Preference was defined by animals spending significantly more time in the drug conditioned chamber after conditioning.

Vehicle animals spent approximately the same amount of time in the S+ chamber before and after conditioning. This indicates that animals do not show compartment bias to the apparatus after repeated conditioning trials. Surprisingly, morphine, our positive control, failed to show place preference. However, the literature on morphine place preference is equivocal. Some labs report place preference across a range of doses whereas others do not (i.e. 1 – 10 mg/kg). Two factors may have played a role in this finding. First, the dose may have been too high (10 mg/kg) as this appears to produce more mixed results than lower doses (for review: Tzschentke, 2007). Secondly, the loss of three data points certainly compromised statistical power. In this study, 1 mg/kg haloperidol produced modest place aversion. The lack of significance was potentially due to too high a dose. The earliest study showing CPA with haloperidol used a much lower dose of 0.1 mg/kg in mice (Risinger, Dickinson & Cunningham, 1992). Thus, haloperidol dosing was lowered to 0.8 mg/kg for the second experiment.

Salvinorin A showed a pattern of place aversion across all doses. This is not surprising as previous research shows salvinorin A producing place aversion (Zhang, Butelman, Schlussman, Ho, & Kreek, 2005). Salvinorin A is a κ-opioid receptor agonist and κ-agonists are known to produce robust place aversion in the CPP paradigm (Mucha & Herz, 1985). *S. divinorum* also produced place aversion approximate to that of salvinorin A. This is not surprising as salvinorin A is the major constituent in *S. divinorum* ("Drugs and Chemicals of Concern: *Salvia Divinorum*,"). These observations suggest that salvinorin A is also the main constituent responsible for the place aversion observed with administration of *S. divinorum*. In previous research, compounds that produce CPA lack abuse potential (Sufka, 1994). Collectively, these findings indicate that both *S. divinorum* and salvinorin A lack abuse potential.

Experiment 2

The goal of the second experiment was to characterize rewarding or aversive properties of *M. speciosa,* a fraction of the botanical, and its active, mitragynine. Following apparatus habituation and quantification of baseline compartment preference, male Sprague-Dawley rats were given eight drug-compartment conditioning trials which involved alternate day pairings of test compound to one compartment and vehicle to the other. This was followed by a compartment preference trial conducted under drug-free states. Preference was defined by animals spending significantly more time in the drug conditioned chamber after conditioning.

Vehicle animals spent approximately the same amount of time in the S+ chamber before and after conditioning. This indicates that animals do not show compartment bias to the apparatus after repeated conditioning trials. Consistent with the literature, d-amphetmine, our

positive control, produced robust place preference (for review see: (Tzschentke, 2007)). Using a slightly lower dose of haloperidol, our negative control, produced marginal place aversion.

Mitragynine shows place preference across doses with robust effects seen at the low and high doses, indicative of abuse potential. This is not surprising, as previous research shows that mitragynine displays affinity to μ-opioid receptors. Compounds with affinity to μ-opioid receptors are known to produce robust place preference (for review see: Hassan et al., 2012). These findings are also consistent with anecdotal evidence suggesting that consumers have difficulty stopping use of the plant (Ahmad & Aziz, 2012).

The whole plant extract of *M. speciosa* shows a pattern of place preference but does not reach significance at any of the doses. The fraction of the plant, however, shows far less rewarding activity than the whole plant extract and mitragynine. In the doses of both the extract and the fraction, the amount of mitragynine mirrors the doses used when testing the active constituent alone. With the amount of mitragynine steady, appearance of significant CPP in mitragynine alone but not in the extract or fraction raises questions. It is suspected that there may be a constituent present in both the whole plant extract and the fraction that is either 1) producing place aversion thus mediating the preference caused by mitragynine or 2) antagonizing the activity of mitragynine.

Future studies

Botanicals typically contain a large variety of constituents that may possess a diverse array of activity. Because of this complexity, the study of botanicals can lead to false negatives when lacking methods that allow for a broad analysis of the plant's psychoactive properties. An example of this problem can be observed in the current evaluation of *M. speciosa*, where results

were not consistent across the whole plant extract, fraction, and mitragynine. The current study utilized a novel approach in which three doses of the entire plant extract, a fraction of the active constituents, and the plant's primary active constituent were tested. This approach allows for a more complete evaluation of the large array of constituents comprising a botanical. Researchers can identify a) if the believed active constituent is wholly responsible for a plant's psychoactive effects, b) if there are further rewarding or aversive qualities in other constituents, and c) if the constituents responsible for alternative qualities are present in the selected fraction. Researchers may want to adopt this 3-tiered approach in future research evaluating botanical products that are suspect for abuse liability and other psychoactive qualities.

Given the varying results of *M. speciosa*, its fraction, and mitragynine, further evaluation of the plant's constituents would make an interesting follow up study. The current findings support evidence of other active constituents with aversive qualities or constituents synergistic to mitragynine. Paynantheine and Speciogynine make up 9 and 7% of the plant, respectively, and may be valuable to investigate. In addition, 7-hydroxymitragynine has been identified as a minor constituent, comprising 2% of the plant, and may be a good option to study (for review see: (Hassan et al., 2013)). Research evaluating alternate constituents of *M. speciosa*, such as 7-hydroxymitragynine, would help researchers to gain a broader understanding of psychoactive qualities of the botanical. If there is a constituent that is not aversive and synergistically interacts with mitragynine's rewarding effects to reduce CPA, this could open new doors in the study of addiction. This constituent could offer insight in attenuating addictive qualities in other drugs of abuse.

LIST OF REFERENCES

Ahmad, K., & Aziz, Z. (2012). Mitragyna speciosa use in the northern states of Malaysia: a cross-sectional study. *J Ethnopharmacol, 141*(1), 446-450.

Assanangkornchai, S., Muekthong, A., Sam-Angsri, N., & Pattanasattayawong, U. (2007). The Use of Mitragynine speciosa ("Krathom"), an addictive plant, in Thailand. *Subst Use Misuse, 42*(14), 2145-2157.

Babu, K. M., McCurdy, C. R., & Boyer, E. W. (2008). Opioid receptors and legal highs: Salvia divinorum and Kratom. *Clin Toxicol (Phila), 46*(2), 146-152.

Bardo, M., Rowlett, J., & Harris, M. (1995). Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neuroscience & Biobehavioral Reviews, 19*(1), 39-51.

Bardo, M. T., & Bevins, R. A. (2000). Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl), 153*, 31-43.

Chavkin, C., Sud, S., Jin, W., Stewart, J., Zjawiony, J. K., Siebert, D. J., . . . Roth, B. L. (2004). Salvinorin A, an active component of the hallucinogenic sage salvia divinorum is a highly efficacious kappa-opioid receptor agonist: structural and functional considerations. *J Pharmacol Exp Ther, 308*(3), 1197-1203.

Clouatre, D. L. (2004). Kava kava: examining new reports of toxicity. *Toxicology Letters, 150*(1), 85-96.

Dennehy, C. E., Tsourounis, C., & Miller, A. E. (2005). Evaluation of herbal dietary supplements marketed on the internet for recreational use. *Ann Pharmacother, 39*(10), 1634-1639.

Drugs and Chemicals of Concern: Kava. Retrieved Febuary 5, 2013, from http://www.deadiversion.usdoj.gov/drugs_concern/kava.pdf

Drugs and Chemicals of Concern: *Mitragyna speciosa*. Retrieved February 5, 2013, from http://www.deadiversion.usdoj.gov/drugs_concern/kratom.pdf

Drugs and Chemicals of Concern: *Salvia Divinorum*. Retrieved February 3, 2013, from http://www.deadiversion.usdoj.gov/drugs_concern/salvia_d.pdf

Griffin, O. H., Miller, B. L., & Khey, D. N. (2008). Legally high? Legal considerations of Salvia divinorum. *Journal of Psychoactive Drugs, 40*(2), 183-191.

Hassan, Z., Muzaimi, M., Navaratnam, V., Yusoff, N. H., Suhaimi, F. W., Vadivelu, R., . . . Muller, C. P. (2013). From Kratom to mitragynine and its derivatives: Physiological and behavioural effects related to use, abuse, and addiction. *Neurosci Biobehav Rev, 37*(2), 138-151.

Hillebrand, J., Olszewski, D., & Sedefov, R. (2010). Legal highs on the Internet. *Subst Use Misuse, 45*(3), 330-340.

Humberston, C. L., Akhtar, J., & Krenzelok, E. P. (2003). Acute Hepatitis Induced by Kava Kava. *Clinical Toxicology, 41*(2), 109-113.

Koob, G. F., Sanna, P. P., & Bloom, F. E. (1998). Neuroscience of addiction. Review. *neuron, 21*, 461-476.

Lüde, S., Török, M., Dieterle, S., Jäggi, R., Büter, K. B., & Krähenbühl, S. (2008). Hepatocellular toxicity of kava leaf and root extracts. *Phytomedicine, 15*(1–2), 120-131.

McCurdy, C. R., Sufka, K.J., Smith, G.H., Warnick, J.E., Nieto, M.J. (2006). Antinociceptive profile of salvinorin A, a structurally unique kappa opioid receptor agonist. *Pharmacology, Biochemistry, and Behavior, 83*(1), 109-113.

Marcus, D. M., & Grollman, A. P. (2002). Botanical Medicines - The Need for New Regulations. *The New England Journal of Medicine, 347*(25), 2073-2076.

Mucha, R. F., & Herz, A. (1985). Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. *Psychopharmacology (Berl), 86*(3), 274-280.

Munro, T. A., & Rizzacasa, M.A. (2003). Salvinorins DF, new neoclerodane diterpenoids from *Salvia divinorum*, and an improved method for the isolation of salvinorin A. *Journal of Natural Products, 66*(5), 703-705.

Pfeiffer, A., Brantl, V., Herz, A., & Emrich, H. M. (1986). Psychotomimesis Mediated by κ Opiate Receptors. *Science, 233*(4765), 3.

Risinger, F. O., Dickinson, S. D., & Cunningham, C. L. (1992). Haloperidol reduces ethanol-induced motor activity stimulation but not conditioned place preference. *Psychopharmacology, 107*(2-3), 453-456.

Sabetghadam, A., Ramanathan, S., Sasidharan, S., & Mansor, S. M. (2013). Subchronic exposure to mitragynine, the principal alkaloid of *M. speciosa*, in rats. *Journal of ethnopharmacology*.

Shellard, E. J. (1974). The alkaloids of Mitragyna with special reference to those of Mitragyna speciosa, Korth. *Bulletin on narcotics, 26*(2), 41-55.

Sufka, K. J. (1994). Conditioned place preference paradigm: a novel approach for analgesic drug assessment against chronic pain. *Pain 58*, 355-366.

Tanguay, P. (2011). Kratom in Thailand. *Kratom in Thailand (April 1, 2011)*.

Teschke, R., Schwarzenboeck, A., & Akinci, A. (2008). Kava hepatotoxicity: a European view. *The New Zealand Medical Journal, 121*(1283), 9.

Tzschentke, T.M. (1998). Measuring reward with the conditioned place preference paradigm: A comprehensive review of drug effects, recent progress, and new issues. *Progress in Neurobiology, 56,* 613-672.

Tzschentke, T. M. (2007). Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol, 12*(3-4), 227-462.

Valdéz III, L. J., Díaz, J. L., & Paul, A. G. (1983). Ethnopharmacology of Ska María Pastora (Salvia divinorum, Epling, and Játiva-M. *J Ethnopharmacol, 7*, 287-312.

Wasson, R. G. (1962). A new Mexican psychotropic drug from the mint family. *Botanical Museum Leaflets Harvard University, 20*(3), 77-84.

Watanabe, K., Yano, S., Horie, S., & Yamamoto, L. T. (1997). Inhibitory effect of mitragynine, an alkaloid with analgesic effect from Thai medicinal plant *Mitragyna speciosa,* on electrically stimulated contraction of isolated guinea-pig ileum through the opioid receptor. *Life Sci, 60*(12), 10.

Whitton, P. A., Lau, A., Salisbury, A., Whitehouse, J., & Evans, C. S. (2003). Kava lactones and the kava-kava controversy. *Phytochemistry, 64*(3), 673-679.

Yamamoto, L. T., Horie, S., Takayama, H., Aimi, N., Sakai, S.-i., Yano, S., . . . Watanabe, K. (1999). Opioid receptor agonistic characteristics of mitragynine pseudoindoxyl in comparison with mitragynine derived from Thai medicinal plant< i> Mitragyna speciosa</i>. *General Pharmacology: The Vascular System, 33*(1), 73-81.

Zhang, Y., Butelman, E. R., Schlussman, S. D., Ho, A., & Kreek, M. J. (2005). Effects of the plant-derived hallucinogen salvinorin A on basal dopamine levels in the caudate putamen and in a conditioned place aversion assay in mice: agonist actions at kappa opioid receptors. *Psychopharmacology (Berl), 179*(3), 551-558.

LIST OF APPENDICES

APPENDIX A

FIGURE CAPTIONS

Figure 1. The effects of *S. divinorum* and salvinorin A on place preference. Values represent mean change in seconds (±SEM) spent in S+ chamber before and after drug-conditioning during a 15 min drug-free test. Solid horizontal line reflects the mean preference score for the vehicle group and is provided for comparative purposes. Scores above baseline signify place preference while scores below baseline signify place aversion. * indicates a significant difference from the vehicle group. Sample sizes were 7-10.

Figure 2. The effects of *M. speciosa*, its fraction, and mitragynine on place preference. Values represent mean change in seconds (±SEM) spent in S+ chamber before and after drug-conditioning during a 15 min drug-free test. Solid horizontal line reflects the mean preference score for the vehicle group and is provided for comparative purposes. Significant conditions that are higher than the baseline signify preference while significant conditions that drop lower than the baseline signify aversion. . * indicates a significant difference from the vehicle group. Sample sizes were 9-10.

APPENDIX B

FIGURES

Figure 1

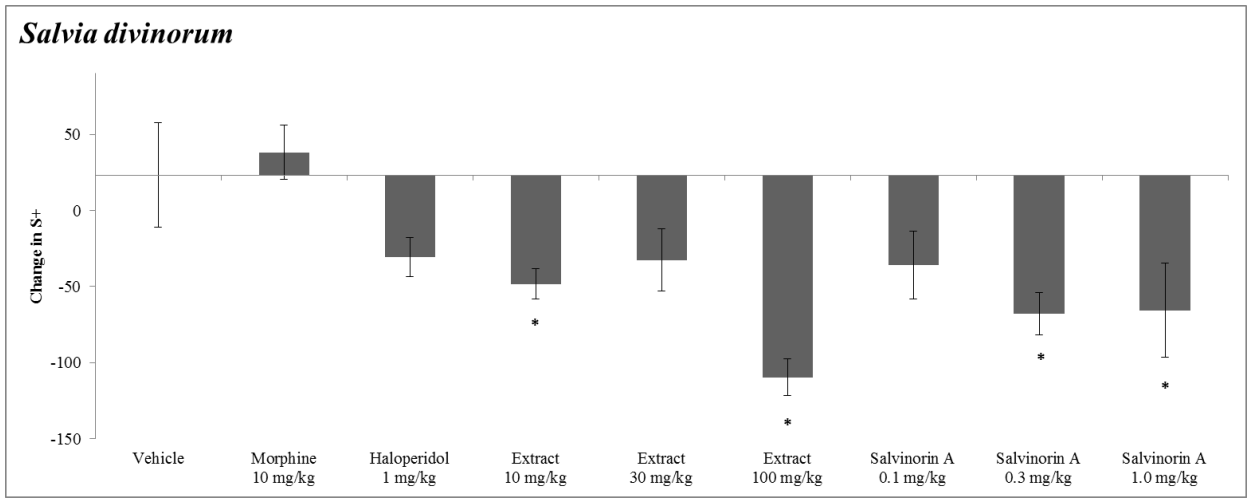
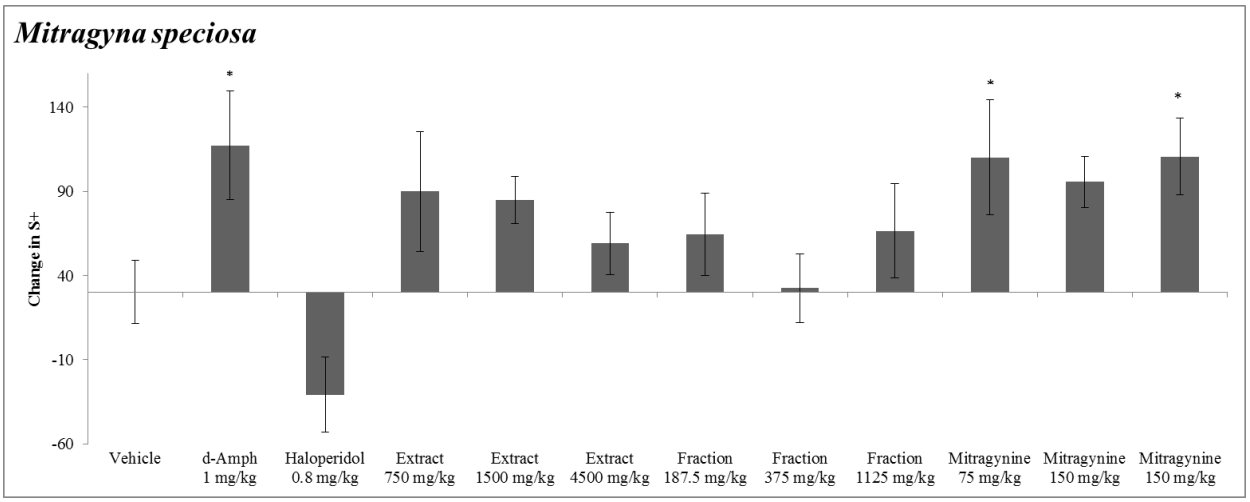


Figure 2



VITA

MELISSA LORIA

458A Highway 334 • Oxford, MS 38655 • (662) 816-5720 • mjloria@olemiss.edu

EDUCATION

August 2011—present	Doctor of Philosophy in Clinical Psychology, Anticipated May 2016, University of Mississippi, Oxford, MS
September 2007—August 2010	Bachelor of Arts in Psychology**,** University of Mississippi

PUBLICATIONS and PRESENTATIONS

Hymel KA, **Loria MJ**, White SW, Salmeto AL, Sufka KJ (June 2013). Strain Vulnerability and Resiliency in the chick anxiety-depression model. 42nd Annual Meeting of the Society for Neuroscience, New Orleans, LA.

Lewellyn KD, Bialonska ND, Chaurasiya BL, Tekwani, BL, **Loria, MJ**, White SW, Sufka KJ, Zjawiony JK (2012). In vitro and in vivo evaluation of the antidepressant activity of aplysinopsin analogs. Planta Medica.

Loria MJ, Harris HM, Lewellyn K, Zjawiony JK, Ali Z, Khan IA, Sufka KJ (April 2013). Evaluating Preference-Seeking and Aversive Qualities of Salvia Divinorum and Mitragyna Speciosa. 12th Annual Oxford International Conference on the Science of Botanicals. University of Mississippi.

Loria MJ, White SW, Robbins SA, Salmeto AL, Hymel KA, Murthy SN, Manda P, Sufka KJ (2013) Brain-derived neurotrophic factor in vulnerable and resilient genetic lines in the chick anxiety-depression model. Behavioral Brain Research.

Loria MJ, White SW, Robbins SA, Salmeto AL, Hymel KA, Murthy SN, Manda P, Sufka KJ (2013) Brain-derived neurotrophic factor in vulnerable and resilient genetic lines in the chick anxiety-depression model. 42nd Annual Meeting of the Society for Neuroscience, New Orleans, LA.

Loria MJ, White SW, Robbins SA, Salmeto AL, Hymel KA, Murthy SN, Manda P, Sufka KJ (2013) Brain-derived neurotrophic factor in vulnerable and resilient genetic lines in the

chick anxiety-depression model. 20th Annual International "Stress and Behavior" Neuroscience and Biopsychiatry Conference, New Orleans, LA.

Salmeto AL, Hymel KA, **Loria MJ**, White SW, Sufka KJ (June 2013). Strain Vulnerability and Resiliency in the chick anxiety-depression model. 20th Annual International "Stress and Behavior" Neuroscience and Biopsychiatry Conference, New Orleans, LA.