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An examination of the consequences of chronic exposure to *Mitragyna speciosa* during adolescence on learning and memory in adulthood

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Abstract

Although an emerging drug of concern in the United States and Europe, the active alkaloids associated the *Mitragyna speciosa* plant have long been utilized for a number of purposes ranging from use as an antitussive to that of anti-inflammatory or analgesic purposes. Known by a number of common names, in the United States it is normally legally sold as Kratom. However, little is known about the consequences of the main constituent, mitragynine or any of the more than two dozen identified plant alkaloids on neuropsychological development, learning and memory, and behavior. In the present experiment, adolescent rats were given repeated injections of saline, 15 mg/kg, or 50 mg/kg of *Mitragyna speciosa* extract. Once the animals reached 107 days of age, they were assessed for general activity, retention on a step-down passive avoidance task, trained using tasks with spatial components of various levels of difficulty, a spatial learning set task, and a plus maze response learning task. In some but not all of the Morris water maze tasks, escape latencies for the 50 mg/kg but not 15 mg/kg rats were significantly longer than that of saline control animals. Nonetheless, performance across groups on probe trials was comparable. In addition, during learning set testing the escape times for the three groups were comparable and, more important, they were able to respond on trial two on the basis of what they learned on the first trial by the end of training. For plus maze response learning testing, all three groups made a comparable number of reference memory errors. Conversely, the 50 mg/kg drug group made significantly more total and working memory errors than the saline-treated animals. The results suggest that chronic exposure to the alkaloids present in legally available Kratom during adolescence is capable of producing a variety of subtle but lasting changes affecting spatial and working memory performance in adulthood, well after the exposure to Kratom has ended.

Keywords: *Mitragyna speciosa*, Kratom, Mitragynine, Spatial Learning, Development, Memory.

Introduction

As a family, *Rubiaceae* include plants of value for landscaping (gardenia, ixora), economically (coffee, blackberries and raspberries, Rose madder), as well as value for their medicinal qualities (e.g., quinine, ipecacuanha).[1] A member of the *Rubiaceae* family, *Mitragyna speciosa* (Korth) is a tree with medicinal properties native to tropical and subtropical regions of Africa and Southeast Asia.[2] *Mitragyna speciosa* is known by a number of names including Kratom, Kakuam, Kraton, Thom, or Ithang in Thailand and Biak-Biak or Ketum in Malaysia.[3-6] In the United States, compounds associated with *Mitragyna speciosa* are usually referred to as Kratom and sold as a legal herb[7,8], although The Vaults of Erowid[9] lists other common names including Ketum, Thom, and Mambog. For the purposes of the present report, Kratom will be used to refer to the phytopharmaceuticals derived from the *Mitragyna speciosa* plant.

In areas of the world where the plant was available, it has been considered useful as a herbal medication to treat a number of problems including diarrhea, coughs, hypertension, and to improve sexual performance.[10-12] More important, *Mitragyna speciosa* was recognized for its properties in the alleviation of pain an effect supported by experimental evidence.[13] More recently, *Mitragyna speciosa* has been used to treat opiate (e.g., heroin) addictions acting as a substitute for the latter in addiction programs.[12-14] Many of the effects of Kratom appear to be

a function of the dose. For example, chewing the leaves of *Mitragyna speciosa* reportedly produces stimulant effects. Conversely, higher levels of Kratom associated with the recreational extracts are reported to produce narcotic analgesic effects.[15] As a consequence of its morphine-like effects, it has been used and is considered a recreational drug.[16] Previous research suggests that chronic *Mitragyna speciosa* users develop an addiction and experience withdrawal symptoms following abstinence.[17] Although the responses are quite varied, Kratom abuse has received increased attention from a number of governments including Thailand and Malaysia[16] as well as the United States, United Kingdom and Germany.[18]

Research has led to the discovery of more than 40 alkaloids associated with Kratom[19,20] with mitragynine and, to a lesser extent 7-hydroxymitragynine, speciogynine and, paynantheine, considered the major alkaloids.[21] Reductions in sensitivity to pain associated with mitragynine appear to be mediated by δ and μ opioid receptors located at the supraspinal level.[6,13,22-24] Binding studies indicated that mitragynine has the highest affinity for μ receptors with somewhat lower affinity for K- and δ receptors.[25]

As of this report, Kratom is still legally available in the United States, although it is listed as a drug with illicit uses and distribution.[26] However, it is under surveillance in the United States, United Kingdom and Germany[18] but Kratom and derivatives are a controlled substance in certain areas of Europe such as Sweden, Denmark, and Poland and regulated by narcotics laws in other countries including Thailand, Malaysia, Myanmar, and Australia.[21] On the other hand, Kratom is both legally grown and exported in Indonesia.[27] Thus, although Kratom has been recognized by a number of countries for its possible adverse effects on health and addictive properties, a consensus in terms of regulations governing relative risk, possession, distribution, and possible benefits has not been achieved.

Although research on the effects of Kratom on behavior and cognitive function is scarce, recently reports have begun to surface. In one study[28], no deficits in motor coordination or short-term memory were reported following oral administration of *Mitragyna speciosa* extract or the alkaloid mitragynine. Conversely, 28 days of i.p. mitragynine exposure impaired working memory performance on an object recognition memory task.[16] Last, methanol extract of *Mitragyna speciosa* at a dose of 1000 mg/kg enhanced acquisition of an active avoidance learning task, an impairment in memory consolidation of a passive avoidance task, and no apparent benefit with long-term memory consolidation. Interestingly, lower (100 & 500 mg/kg) doses did not influence performance.[29]

Research conducted on human participants is just as limited and is usually conducted in non-treatment settings. For example, assessment of Kratom users revealed no problems in short or long-term memory, logical reasoning, or executive function. On the other hand, deficits in visual-spatial perception were found.[30,31] However, given the nature of these studies and others, adequate experimental designs with appropriate dose-response estimates are necessary before any conclusions can be drawn.[21]

Adolescence in rats lasts from the 21st postnatal day following birth until 60 days of age.[32,33] Rat adolescence can be further defined in terms of mid adolescence and late adolescence (34 to 46 and 46 to 59 days old). These two periods are considered as analogous to periadolescence and late adolescence/early adulthood, respectively.[33] Thus, an adolescent rodent model is useful for both comparative evaluations and for extrapolation to humans.[34] Specifically, the use of adolescent animals provides a valuable experimental framework for an examination of the developmental consequences associated with exposure to compounds at various points in biological and cognitive development.

To reiterate, the available scientific information about the effects of chronic exposure of Kratom on the central nervous system is inadequate.[21] It is important to acknowledge the possibility that Kratom may have therapeutic uses such as a substitute for opiates[10,12,35], as an anti-inflammatory[35,36], and as an anti-depressant.[6,37] However, given the growing popularity of Kratom, the possible risks on development in vulnerable adolescents and young adults could become a major concern, especially as a potential societal health problem. In addition, there are no published studies on the long-term effects of developmental exposure to the Kratom compounds on the physiology of learning, and memory. Therefore, the present study was conducted to examine the influence of repeated adolescent exposure of Kratom on learning and memory performance in Morris water maze (MWM) tasks of varying difficulty. Specifically, drug-free adult animals were trained on a cued version of the MWM to evaluate whether nonassociative factors influenced place learning performance. A series of non-cued MWM tasks of varying difficulty were used to evaluate learning, memory, and via probe trials, retention. Finally, a simple response set learning task was employed to explore possible response perseveration and memory deficits.

Materials and Methods

Plant materials

Kratom (*Mitragyna speciosa*) product was purchased from the Internet through the http://www.ethnobotanicals.com website. According to a declaration from the provider, the shredded leaves of M. speciosa originated from Thailand. Adapting methods described elsewhere[36], a mitragynine alkaloid extract was isolated. The resulting mitragynine was dissolved in 0.5% (v/v) Tween80 (Sigma–Aldrich, USA). Doses of 15 mg/kg or 50 mg/kg were chosen after examination of scant existing literature as well as pilot testing with rats not included in the present study. In the present study, we used two drug groups consisted of eight animals each with eight rats serving as a control group. Rats in the control group received injections consisting of an equivalent volume of isotonic saline solution. All injections were delivered IP.

Analysis of the characteristics of the purchased Kratom sample was performed using HPLC following a modification of the method described elsewhere.[38] The analysis was performed using a Waters XTerra C18 4.6 mm x 250 mm, 5 μm column (Milford, MA, USA). The mobile phase, with a MeCN/0.01% ammonia solution (70:30, v/v) included the following. The detection wavelength was λ 254 nm, pH 10.3, injection volume

10 mL, flow rate 1 mL/min, with a total time of analysis was 20 minutes.

Experimental animals

Twenty-six male experimentally naive Sprague-Dawley rats (Charles River, Wilmington, MA) served as the subjects. Two rats died during the course of the experiment and therefore were not included in any of the analyses. The research protocol was approved by the Institutional Animal Care and Use Committee of Palm Beach Atlantic University and all animals were cared for according to the principles outlined in the Guide for the Care and Use of Laboratory Animals.[39] After a one-week acclimation period in the laboratory with daily handling, the rats were assigned to one of three groups. As noted above, two groups receiving daily injections of either 15 mg/kg or 50 mg/kg of mitragynine. Drug exposure began when all of the rats were 35 days of age and continued daily for a period of 30 days.

Throughout the experiment, the rats were individually housed in stainless steel suspended cages and maintained on a 12-hr light/12-hr dark cycle with the lights on at 7:00 am. The animals were provided with *ad libitum* access to water and food (Mazuri Rodent Chow).

Apparatus – Morris water maze (mwm)

All spatial tests took place in different configurations in a circular white acrylic plastic swimming pool with a diameter of 183 cm. With the exception of the cued water maze phase of the experiment (see following), the depth of the water was held constant at 30 cm and made opaque white in color using a nontoxic water-based paint (Sargant Art, Hazelton, PA). The swimming pool was located in a quiet testing room approximately 36.88 square meters in size. Through the use of white curtains, a minimum number of external stimuli were available to aid navigation when viewed from the surface of the pool. Throughout all phases of training and testing, with the exception of free swim "probe" trials, a 15 cm X 15 cm flat white escape platform was used. For the cued water maze task described below, the platform extended 15 mm above the surface of the water and was located 18 cm from the wall of the swimming pool. For all other phases of the experiment, the escape platform was submerged to a depth of 15 mm below the surface of the water.

Experimental procedures

The series of behavioural tests begin 42 days following the final drug exposure. The purpose of our behavioural testing regimen was to ascertain whether chronic mitragynine exposure produced lasting cognitive deficits and if present, to tentatively assess the severity of such deficits. First, a test of general activity levels and a cued version of the MWM were administered to provide a general assessment of the physical state of the rats. Spatial learning and memory was evaluated using variations of standard MWM navigation tasks of varying difficulty. Last, a nonspatial response learning was tested using a Morris water maze version of a plus maze task described in greater detail elsewhere.[40]

Assessment of general activity and exploration- General activity and exploratory levels were evaluated for 5 minutes in a 24" x 24" chamber consisting of a series of 6" squares (i.e., a checkerboard). The general activity of each rat was determined by the number of squares crossed during the measurement period. The number of times the animals rose onto their hind legs was also tallied. In addition, motivational and sensorimotor deficits were assessed using a cued version of the MWM task described below.

Step-down passive avoidance tests- The step-down passive avoidance testing occurred in a standard operant chamber (Lafayette Model 84022) with a stainless steel electrified grid floor. Located in the centre of the 21-cm x 28-cm chamber was a 10.14-cm x 10.14 cm brick block. A 0.4mA current was delivered to the feet of the rat whenever the animal left the block and touched the grid floor.

Water maze tasks

The series of water maze protocols we employed permitted us to assess facets of rodent learning and memory without the need for food deprivation associated with appetitive tests of memory. For all MWM elements, the platform was either 15 mm above the water's surface (cued memory task) or was submerged 15 mm below the surface of the water (the place & spatial learning set tasks). Across all spatial phases of the experiment, on a given trial the rat was released into the pool at one of four release points, labeled north, south east, or west, and allocated 60 seconds per trial to find the escape platform. The platform location varied at one of four compass positions – northeast, northwest, southeast, or southwest. If the rat was unable find the platform within the allocated period, it was gently placed on the platform. Escape times to the escape platform were recorded with a stopwatch and errors, operationally defined as crossing one of four quadrants associated with the four compass points, were tabulated by no fewer than teams of two experimenters.

Cued water maze task- Following a drug recovery period, the assessment of general activity and passive avoidance testing, the animals were tested a cued version of the MWM. The cued version of this navigation task allowed for the determination of whether performance factors unrelated to the place learning elements in later test phases were present.[41] Specifically, through the use of a visible platform, we were able to test for the presence of sensory (visual), motor (swimming ability), and motivational deficits as well as nondeclarative memory problems that could adversely impact performance during the later phases of the study. Training on the cued water maze navigation task began when the rats were 110 days old, 45 days after the last drug exposure. The rats were given 10 trials per day for 2 days. Rats were allowed to remain on the platform for 15 seconds after each trial.

Place learning water maze tasks- Two variations of MWM place learning tasks were used during this phase of testing. Both are considered tests of spatial reference memory that differed in terms of difficulty. The place learning tasks involved learning the location of a submerged platform that remained constant across all trials within a given phase of the experiment. Two variations of the task were used because often no or only minor deficits are reported using the standard version of this test.[42] Therefore, a more difficult version of the place learning task was included as the latter of the two was considered more sensitive for detecting spatial

learning/memory impairments following chronic exposure to mitragynine.

The high cue version of the place learning task consisted of training the rats for 10 trials per day for 2 days. The rats were allowed to remain on the platform for 15 seconds after each trial. A test of retention was evaluated with a probe trial on the second day. This consisted of removal of the escape platform and testing the subject on a 60 second ''free swim'' approximately 2 hours after the last place learning trial. Both the time spent swimming in the target quadrant and the number of crossings over the former platform location were recorded.

With fewer external cues available to aid navigation, this task was considered more difficult. For the low cue version of the place learning task, the rats were trained 4 consecutive trials per day for 5 consecutive days. As in previous MWM phases, task difficulty was increased by placing a curtain around the water maze. In addition, during this phase task difficulty was increased by indirectly lighting the room with a single 60 watt red light bulb located beyond the curtain and approximately 3 metres from the water maze. As a result, few visual cues remained to aid navigation. Rats were allowed to remain on the platform for 15 seconds after each trial. Daily probe trials were administered 2 hours after the last trial of the daily four-trial series.

Spatial learning set acquisition testing- The learning set acquisition phase required the rats to learn the new location of the escape platform on each day of testing. This phase of testing required flexible responding in the face of changing environmental demands. Further, the performance on Trial 2 of each day was defined as an index of spatial working (short-term) memory because the animal was required to recall its response on the trial immediately preceding the current one. All animals received 4 consecutive trials per day with the testing continuing for 5 consecutive days, on post-drug exposure days 54 to 59. The rats were allowed to remain on the platform for 15 seconds at the completion of each trial.

Nonspatial response learning- The last phase of the experiment involved the use of a plus maze response learning task similar to that discussed elsewhere.[40] Here, the goal was to assess nonspatial response learning as well as perseverative behaviour. In the task, the animal was faced with three response alternatives - to turn either left or right, or to swim straight ahead. Using a Fellows series[43], the order in which the animals were placed at one of the two starting points was randomly chosen. Consistent with all earlier tests, the trials in this phase began by lowering the animal to the surface of the water facing the wall of the tank. As a result, the animal was required to turn 180° and swim towards the choice point at the center of the plus maze.

As noted by McDaniel and colleagues[44], within a given set of trials, the configuration of the available allocentric information differed as a function of each trial. Therefore, in order to master the task, (i.e., "turn right vs. left"), the animal was required to learn a rule to turn in a specific direction regardless of the starting location. While the goal remained fixed for each animal and a series of reversals were not considered here, the ability to flexibly adjust behaviour as a function of available allocentric cues has proven to be an effective measure of perseverative behaviour.

Data analysis

For all MWM tasks, the plus maze response learning task, and step-down passive avoidance, escape time and navigation errors (if relevant; maze tasks only) were the two primary measures of performance. For the plus maze phase of the experiment, total errors were divided into working and reference memory errors (see below, nonspatial response learning section).

Across the MWM tasks, the optimal swim path distances differed depending on the start and escape loci. Therefore, the recorded escape latencies for the four start locations were normalized. Normalization involved computation of the ratio of the minimum swim distance in cm for each of the two longer swim paths to the escape platform (e.g., south start location and a northwest goal location) to the minimum swim of the two shorter swim paths (e.g., south start location and a southwest goal location) trials in cm.

Statistical analyses involved mixed analysis of variance (ANOVAs), with drug group as the between-subjects factor and days, or blocks of trials and days as within-subjects factors. Post-hoc analyses were performed using $Tukey_{HSD}$ or paired *t*-tests with a Bonferroni correction to control for multiple comparisons. The alpha level for acceptance was set at $p < .05$ and analyses were performed using SPSS ver. 22.

Results

General activity

Assessment of the activity data revealed no group differences in the number of squares traversed, or in the number of rearing.

Step-down passive avoidance testing

Analysis of the step-down avoidance data was performed using a 3 (drug groups) X 2 (trials) mixed ANOVA. No differences in step-down latencies as a function of group were observed. A main effect of trials was detected, $F(1, 19) = 31.44$, $p < .001$, $\eta_p^2 = .601$, suggesting that the animals retained the memory of the adverse experience from the first day (Day 1: $M = 10.38$, $SD = 4.70$; Day 2: $M = 32.67$, $SD = 19.07$). Nonetheless, the drug group X trial interaction was nonsignificant, indicating that group latencies were comparable across trials.

Cued water maze task

The assessment of cued navigation performance was performed using a 3 (drug groups) X 2 days X 2 (blocks) of trials mixed ANOVA, with the latter two variables treated as within-subjects variables. Cued spatial navigation in the Morris water maze presented no difficulty for any of the groups. Only the main effects days, $F(1, 19)$ 47.73, $p < .001$, $\eta_p^2 = .715$, blocks of trials, $F(1, 19)$ 18.80, $p < .001$, $\eta_p^2 = .497$, and the days X blocks interaction, $F(1, 19) = 13.34$, $p < .01$, $\eta_p^2 = .412$, were significant. Visual inspection of the mean latencies to the escape platform suggested a predictable decrease as a function of experience.

The high cue version of place learning

Examination of the escape latency data from the high cue place learning phase revealed the following. Analysis of the data

with a 3 (drug groups) X 2 (days) X 2 (blocks) mixed ANOVA indicated significant within-subject main effects of days, $F(1, 19) = 8.59$, $p < .001$, $\eta_p^2 = .311$, and blocks of trials, $F(1, 19) = 13.40$, $p < .001$, $\eta_p^2 = .414$, suggesting that escape latencies generally improved as a function of training. In addition, a significant main effect of drug group was found, $F(2, 19) = 5.29$, $p < .05$, $\eta_p^2 = .358$. Closer examination of this result using $Tukey_{HSD}$ revealed that across this phase of the experiment, escape latencies were significantly greater for the 50 mg/kg Kratom-treated animals than for the saline-treated control rats. The escape latencies for the 15 mg/kg Kratom-treated animals were similar to those of the saline treated rats. However, the comparison of the 15mg/kg and 50 mg/kg Kratom groups was nonsignificant. Last, the lower-order and three-way interactions were all nonsignificant.

Consideration of the quadrants crossed and time spent in the former location of the escape platform using a one-way multivariate analysis of variance (MANOVA) revealed the absence of a drug effect on both dependent measures. Thus, all animals spent a comparable amount of time in the former target quadrant and explored a comparable number of quadrants across the probe test period.

The low cue version of place learning

In order to analyze the data associated with this more complex place learning task, all trials were normalized and the four daily trials averaged. Inspection of the resulting data with a 1-Between (drug groups), 1-Within (days) ANOVA, the analysis revealed a main effect of drug group, $F(2, 19) = 6.16$, $p < .01$, $\eta_p^2 = .393$, but not days of testing (see Fig. 1, Panel A). The drug group X days interaction was also nonsignificant. Subsequent $Tukey_{HSD}$ tests indicated that, much like that of the high cue place learning task, escape times differed between the control group and 50 mg/kg Kratom rats. Once again the escape times of the 15 mg/kg Kratom-treated animals were intermediate between but not significantly different from either the other two groups.

When the probe trials were analyzed, a different pattern from the result reported in the last paragraph emerged (see Fig. 1, Panel B). For example, the main effect of drug groups was nonsignificant as was the drug group X days interaction. However, a significant main effect of days was detected, $F(4, 76) = 72.69$, $p < .001$, $\eta_p^2 = .793$. Pairwise decomposition of this result with Tukey tests indicated that collectively the rats spent significantly less time in the target escape quadrant on days two and three than on days one, four, and five. The time spent in the target quadrant was similar for the latter three. Thus, although probe performance varied across test days, the search patterns of all groups were similar.

Spatial learning set acquisition testing

The escape time data associated with the spatial learning set task is presented in Fig. 2. To facilitate the interpretation of the data, the first and last days of testing were considered. Within these days, only trials one and two were compared. A 1-between, 2-within mixed ANOVA revealed the following. The main effect of drug group and was nonsignificant. The main effect of day of training, $F(1, 19) = 19.76$, $p < .001$, $\eta_p^2 = .510$, and trials, $F(1, 19) = 14.77$, $p < .001$, $\eta_p^2 = .437$, were significant. These results suggest that the escape times for the three groups were comparable and, more important; they were able to respond on trial two on the basis of what they learned on the first trial by the end of training. In addition, a groups X days interaction, $F(2, 19) = 8.59$, $p < .01$, $\eta_p^2 = .475$, was found as well as a group X days X trials interaction, $F(2, 19) = 3.53$, $p < .05$, $\eta_p^2 = .282$. Decomposition of the interaction by closer examination of trial one versus trial two performances revealed the following. By day five of testing, paired t-tests for each group indicated that escape times were significantly shorter on trial 2 than trial one, smallest $t(5) = -9.68$, $p < .001$. Conversely, on the first day of training, trial one and trial two escape times were comparable.

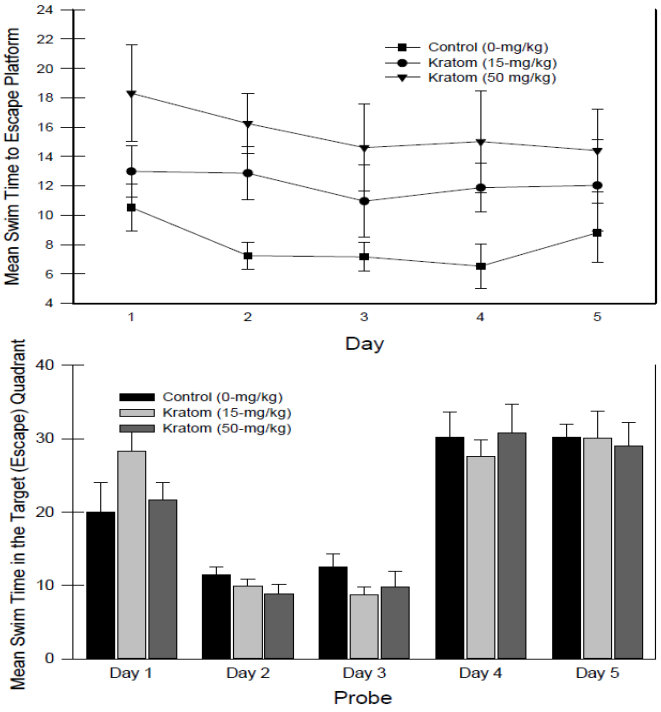

Figure 1: The low cue version of place learning place learning results. The top panel reflects escape performance over the 5 test days. The bottom panel is a presentation of the time spent in the target quadrant during probe testing. Vertical bars represent the standard error of the mean.

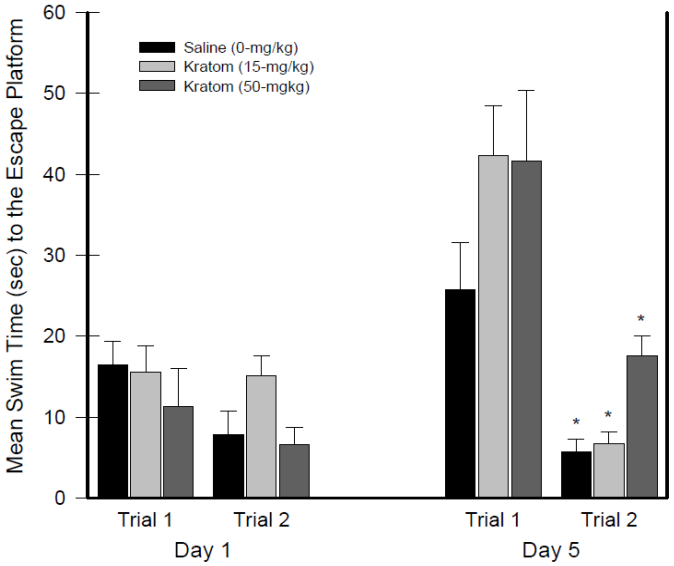

Figure 2: Bar graphs representing the mean and standard error of the mean (vertical bars) of trial one versus trial two performances on the learning set task at the beginning and end of testing. *Significant difference between trial one and trial tow escape latencies ($p < .05$).

Nonspatial response learning

Following spatial learning set testing, training in a plus maze version of the standard MWM began. Daily training involved ten trials per day, continuing five days per week until a criterion of nine errorless escape trials occurred within a single training session or a ceiling of 100 total trials was reached. Consistent with the framework described elsewhere[44], total errors were scored as either working or reference memory errors. Reference memory errors were scored whenever an animal initially entered an incorrect alley while working memory errors were defined as re-entries into incorrect alleys. As defined in our study, working memory errors are considered indicative of a problem in perseverative behaviour. The resulting data were analyzed using a one-way MANOVA, with the measures of working memory, reference memory, and total errors serving as the dependent measures.

Consideration of the data revealed the following. The overall MANOVA was significant, Wilk's $\lambda = .684$, approximate $F(6, 36) = 3.12$, $p < .05$, $\eta_p^2 = .342$. As can be seen in Fig. 3 (panel C) a significant drug effect was observed for total errors, $F(2, 19) = 7.04$, $p < .01$, $\eta_p^2 = .425$, as well as working memory, $F(2, 19) = 8.81$, $p < .01$, $\eta_p^2 = .481$, errors (see Fig. 3, panel A). Conversely, all three groups made a comparable number of reference memory errors. Post hoc examination of the drug effect using Tukey tests indicated the 50 mg/kg Kratom had significantly higher levels of total and working memory errors than the saline-treated animals. As seen in Fig. 3 errors in the 15 mg/kg Kratom groups were intermediate between those of the other two groups but nonsignificant.

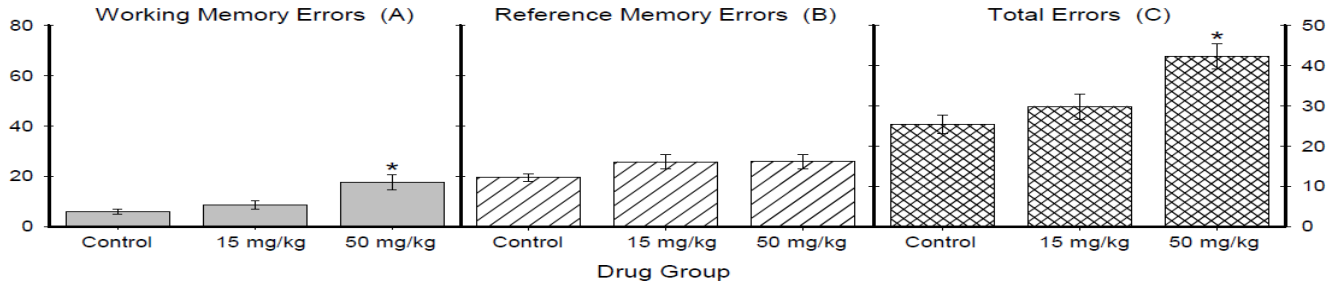

Figure 3: Graphical representation of the mean and standard error of the mean for (a) working memory errors, defined as re-entry errors, (b) reference memory errors, defined as initial alley entrance errors, and (c) total errors. Notes. *Significantly different from the saline control group ($p < .05$).

Mitragynine verification

Examination of the HPLC chromatogram revealed a peak consistent with that of mitragynine. Although somewhat different the sample retention time (10.741) was comparable to that reported elsewhere (i.e., 10.77).[38]

Discussion

Compounds acting as opiate agonists and antagonists may have a marked impact on memory. The mammalian central opioid system is implicated in a variety of functions within the central nervous system[45], including learning, memory, and cognition.[46-48] For example, there is considerable evidence that endogenous opioids and opioid receptors are implicated in both the induction as well as the modulation of hippocampal plasticity.[49-53] Current evidence suggests that different opioid receptors are involved in the modification of synaptic plasticity[52,54], with μ opioid receptors centrally involved in spatial learning and memory.[55] Further, there is convincing evidence that administration of intrahippocampal opioid agonists compromises performance in spatial memory tasks[56,57]. While purely speculative, it is possible that, where found, the memory impairments observed here were the result of altered opioid activity in the hippocampus.

Adolescence is a period substantial neural development as well as behavioral growth and change.[58,59] In fact, the corticolimbic brain regions including those necessary for appropriate and flexible responding on the basis of environmental information are among the last brain circuits to reach maturity in humans and in rodents.[60,61] Among these, areas such as the prefrontal cortex, nucleus accumbens, and basolateral amygdala mature late in brain development.[62,63] Therefore, since adolescence is such a programmed period for of the neural change associated with maturation, it is not unreasonable to posit that this period of development is quite sensitive to any number of external influences (e.g., drugs, environment) that have the ability to induce plasticity in the nervous system.[63]

The period of periadolescence[33], usually defined as rodent mid-adolescence or post-natal days 34 to 46, is a period marked by considerable maturational changes including neuronal development.[58] Specifically, periadolescence includes a large number of developmental changes across neurochemical, metabolic and hormonal domains[58], all of which are responsible for any number of documented behavioral characteristics.[64] Thus, the timing of exposure to drugs can lead to behavioral differences between animals exposed during adolescence as opposed to those exposed later in life. Such effects are seen in tests involving exposure to variety of compounds including ethanol[34,65], nicotine[66], MDMA and MeO-DIPT[40], cocaine[67], and opiates.[64] Adolescent exposure to drug compounds can produce effects that last well into adulthood[32,34,40,68] Perhaps more disturbing, adolescent drug exposure may produce a marked increase in vulnerability to the effects of drugs of abuse in adulthood.[64]

Unfortunately, although considerable research is available concerning the detrimental effects associated with opiate expsusre[69,70], few studies have examined the negative impact of opioids as a function of age of exposure.[64] There is evidence that the abuse of opiates can result in a disruption in neurocognition.[71] In work with human subjects, individuals who have become dependent on opiates have evidence of a loss of volume in the cortex, with decreases in gray matter density in both the temporal and prefrontal cortices.[72,73] Similar

reductions are observed in children with in utero exposure to heroin.[74]

The present results suggest that, at least at the present dose, Kratom is capable of producing working memory and some subtle spatial navigation deficits following adolescent exposure. As such, although the timing of exposure and subsequent test period are different from others, the results are consistent with recent research reports concerning deficits in cognition following acute or chronic exposure of mitragynine.[16,29]

However, certain issues remain before definitive conclusions can be drawn. For example, one of the major issues associated with the consumption of commercially available herbal-highs such as Kratom as well as systematic exploration of their effects is the widespread use of synthetic additives to the plant material.[38] Further, mitragynine is an alkaloid identified exclusively in *Mitragyna speciosa*. Unfortunately, advertised Kratom products are often "enhanced" by either soaking or spraying the leaf material with synthetic narcotics. For example, in Sweden a product sold under the name Krypton was attributed to the deaths of nine individuals.[75,76] The Kratom product identified actually consisted of leaves of *Mitragyna speciosa* and o-desmethyltramadol[75,76], both acting as μ-opioid agonists and causing respiratory depression.[38] Last, occasionally non-narcotic plant materials are treated with narcotic compounds.[77]

Although firm conclusions are premature, research by Senik and colleagues[78] is instructive. Senik *et al*. found that methanolic *Mitragyna speciosa* extract prevented the formation of LTP, while inducing short-term potentiation. A marked reduction in field EPSPs was also found. Such results are informative, because long-term potentiation (LTP) has long been considered the cellular mechanism associated with a memory processes including working memory. Briefly, LTP in the hippocampus and in several areas of the brain, is dependent on presynaptic release of the neurotransmitter glutamate.[79] NMDA glutamate receptors control calcium channels that only open if the cellular membrane is already depolarized.[80] It is a combination of membrane depolarization via input from synaptic connection and of glutamatergic NMDA receptors that permits the entry of Ca^{2+} into the intracellular fluid, which in turn activates AMPA receptors, ultimately leading to alterations in the shape of the dendritic spine and the growth of new dendritic spines.[81,82] For longer-lasting forms of LTP, protein synthesis is required. Ca^{2+} ions activate the CaM-KII enzyme, deactivating the Pin1 enzyme and preserving the production of PKM-zeta.[83] In turn, PKM-zeta causes AMPA receptors to continue movement to the membrane while simultaneously inhibiting Pin1.[84]

The results are suggestive that the consequences of adolescent exposure to Kratom include but, are not necessarily limited to, a long-term impact on learning and memory. As noted earlier, during adolescence, a number of areas of the brain are undergoing developmental changes. Higher levels of novelty and sensation-seeking are considered common in adolescence.[32] However, whether the effects reported here are directly due to mitragynine in the crude extract or one of the other psychoactive alkaloids associated with Kratom remains to be determined. In fact, no information is available to resolve the basic question as to whether it is mitragynine or another psychoactive component of *Mitragyna speciosa*[85] that drives the effects reported here as well as those reported elsewhere.[16,85,86] At any rate, the results reported here should be considered preliminary. As noted earlier, during adolescence, a number of areas of the brain are undergoing developmental changes. Adolescence is a time marked by higher levels of novelty and sensation-seeking.[32] Certainly the data presented here as well as others[16] raise the possibility the mitragynine or another constituent of *Mitragyna speciosa* could, via binding at opioid sites, interfere with such processes as glutamatergic and GABAergic neurotransmission and LTP. Given the availability of the Kratom, the drug should be examined in greater detail, especially among a teenage population at risk for the possible consequences associated with its use.

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