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Article

Behavioral characterization of ayahuasca treatment on Wistar rats in the open field test

Cid Pinheiro Farias[1], Paula Pólvora Victoria[2], Janaína Xavier[2], Fernanda Gelati Sekine[2], Eduardo Silveira Ribeiro[3], Giana de Paula Cognato[2], Hudson Wander Carvalho[1*]

[1]Faculdade de Medicina, Psicologia e Terapia Ocupacional, Curso de Psicologia, Universidade Federal de Pelotas, Pelotas, RS, Brasil, [2]Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, RS, Brasil, [3]Programa de Pós-graduação em Biotecnologia, Universidade Federal de Pelotas, Pelotas, RS, Brasil

Ayahuasca (AYA) is a psychedelic beverage with therapeutic potential for many mood and anxiety disorders. Although there are some preclinical studies, no published reports have tested the behavioral effects of AYA gavage in animal models. This investigation aimed to characterize the behavior of Wistar rats after acute ingestion of AYA for 40 min in the open field test (OFT). The sample consisted of three experimental groups treated with different dosages of AYA (125, 250, or 500 mg kg^{-1}) and a control group. Each group consisted of 10 participants. After gavage, the number of crossings of the OFT grid lines, latency to enter the central area of the device, grooming frequency, and time spent in the central perimeter of the device were immediately evaluated. Analyses were based on one-way ANOVA and a linear-regression mixture model for longitudinal data. AYA intake did not interfere with habituation. The 500 mg kg^{-1} group showed a decrease in the time spent in the center of the device and in the number of crossings compared to the control group in the last 10 min. These results suggest that gavage with AYA did not interfere with the results, and the behavioral effects were perceived only between 30 and 40 min after gavage. Taken together, the results indicate that three aspects should be considered in OFT studies of AYA acute effects: the moment when the observation starts, the observation period, and the AYA dosage.

Keywords: Ayahuasca. Rat model. Open field test. Behavioral tests.

INTRODUCTION

Ayahuasca (AYA) is a pre-Colombian entheogenic tea originally used in shamanic rituals by the Amazon Amerindian population for combined spiritual and medical purposes (McKenna, 2004). In recent decades, it has also been used in neo-shamanic rituals by the urban population in South America and western countries (Labate, Feeney, 2012). Robust evidence from heterogeneous studies indicates that AYA use may enhance psychological well-being and prevent—or even treat—mood, anxiety, and substance-use disorders (Carbonaro, Gatch, 2016; Dos Santos *et al.*, 2016; Palhano-Fontes *et al.*, 2019).

Most commonly, AYA is produced from the decoction of two Amazonian plants: *Banisteriopsis caapi* and *Psychotria viridis*. The first has plentiful β-carbolines such as harmine, tetrahydroharmine, and harmaline, which have potent monoaminoxidase-inhibiting effects (McKenna, 2004). The second has a significant quantity of *N*,*N*-dimethyltryptamine (DMT), a serotonin agonist (5-HT1A/2A/2C) with powerful hallucinogenic properties (Carbonaro, Gatch, 2016).

The mechanism of action of these β-carbolines and DMT involves the inhibition of monoaminoxidase, which simultaneously increases levels of endogenous

***Correspondence:** H. W. de Carvalho. Faculdade de Medicina. Universidade Federal de Pelotas. Avenida Duque de Caxias, 250, CEP 96030-001. Pelotas, Rio Grande do Sul, Brasil. E-mail: hudson.carvalho@ufpel.edu.br.

monoamine neurotransmitters such as serotonin and enables the action of DMT in the central and peripheral nervous systems (McKenna, Towers, Abbott, 1984). These neurotransmission pathways have been associated with many vegetative and neuropsychological functions (Bacqué-Cazenave *et al*., 2020). Furthermore, data indicate that enhanced serotonin neurotransmission is associated with a reduction in inflammation and oxidative parameters in the brain (Gałecki, Talarowska, 2018; Wu *et al*., 2019). In fact, converging evidence shows that the therapeutic potential of AYA arises from the prevention of low-grade inflammation and oxidative stress in the organism, particularly in the nervous system (Frecska, Bokor, Winkelman, 2016). Some evidence has emerged from studies that used the open field test (OFT) paradigm to observe the behavioral and pharmacological effects of AYA (Pic-Taylor *et al*., 2015).

The OFT is one of the most commonly used procedures in animal psychology to assess exploratory and locomotor behavior, anxiety-like manifestations, and general activity in rodents. Many preclinical psychopharmacological studies rely on the OFT to evaluate the effects of specific substances on behavioral and biological parameters. In the OFT, animal behavior is assumed to be a treatment variable, rather than a function of other uncontrolled/intervening variables. To fulfill this assumption, testing protocols prescribe a pre-test period of acclimatization or habituation of the animals in the apparatus. In addition, habituation aims to control neophobic responses and establish baseline thigmotaxis, increasing the accuracy and validity of the test (Prut, Belzung, 2003; Gould, Dao, Kovacsics, 2009). Some studies performed habituation on the device days before testing, while others followed habituation with immediate testing. Operational heterogeneity in habituation times, testing times, and dosages has been observed in animal studies involving acute AYA administration (Lima *et al*., 2007; Oliveira-Lima *et al*., 2015; Pic-Taylor *et al*., 2015). Currently, many studies have evaluated animal behavior under the effect of AYA, but there is a complete absence of studies evaluating the effect of acute AYA treatment on baseline behavior. Furthermore, the methodologies of published behavioral studies of acute AYA effects are heterogeneous; there is no clear determination of the concentration of AYA, or of the after-gavage delay, required to produce salient behavioral effects (Nunes *et al*., 2016; Dos Santos *et al*., 2016; Dos Santos, Hallak, 2017).

For the purposes of behavioral characterization, it is therefore important to consider possible effects of the AYA gavage procedure and ingestion on the habituation of animals. Aversive taste, malaise, vomiting, and diarrhea have been reported in association with AYA consumption (Kjellgren, Eriksson, Norlander, 2009); these may interfere with habituation and other behaviors of experimental subjects (Gould, Dao, Kovacsics, 2009).

Thus, the current investigation aimed to characterize some behavioral patterns of adult Wistar rats fed with three distinct doses of AYA during a 40 min observation period in the OFT. The initial hypotheses motivating this study protocol were as follows. (1) Gavage with AYA, its residual taste, and possible effects contiguous to ingestion of AYA (including nausea) will not affect the habituation of animals in the apparatus to the extent of neophobia or other observable behavioral alterations. (2) Considering that the onset of AYA effects in humans tends to begin around 1 h after consumption (Brito-da-Costa *et al*., 2020), and that the basal metabolic rate of rodents is six to seven times faster than that of humans, a 40 min observation period will be sufficient to identify the onset of AYA effects on behavior. (3) The higher the AYA dosage, the greater will be the behavioral alteration, in a linear dose-dependent relationship.

MATERIAL AND METHODS

This study followed both the animal-care protocols of the official government guidelines and the Institute of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 8023, revised in 1978). The design of the study was approved by the Ethics Committee for Animal Experimentation of the Federal University of Pelotas (Brazil; UFPel-CEEA) and was approved under file number 5151.

The three liters of AYA used in this research was donated by the *Santo Daime* church in March 2017. Characterization of the main psychoactive compounds in our AYA sample followed the protocol described by

Xavier *et al.* (2021). Briefly, DMT and beta-carbolines were extracted from an AYA sample and quantified using gas chromatography with a nitrogen–phosphorus detector through positive standards of each AYA alkaloid.

The concentrations of psychoactive alkaloids present in a dosage of 500 mg kg^{-1} of AYA were 0.28 mg kg^{-1} of DMT (0.056% w/w), 0.70 mg kg^{-1} of tetrahydroharmine (0.14% w/w), 0.13 mg kg^{-1} of harmaline (0.026% w/w), and 0.57 mg kg^{-1} of harmine (0.114% w/w).

A sample of 40 male Wistar rats was equally and randomly divided into a control group to be gavaged with water and three experimental groups to be gavaged with 125, 250, and 500 AYA mg kg mL^{-1} (Castro-Neto *et al.*, 2013; Xavier *et al.*, 2021), respectively. The animals were between 30 and 50 days old and weighed between 200 and 500 mg. Therefore, three suspensions of AYA—125 mg mL^{-1}, 250 mg mL^{-1} and 500 mg mL^{-1})—were used to perform the gavages of each group according to the weight of the animals. For example, if a rat in group 125 weighed 250 mg, it was gavaged with 250 μL of the 125 mg kg^{-1} mL^{-1} AYA suspension.

After AYA treatment, animals were subjected to the OFT paradigm for 40 min, and behavioral observations were clustered and compared based on four periods: 0–10, 10–20, 20–30, 30–40 min (Oliveira-Lima *et al.*, 2015). Latency to enter the central area of the apparatus was assessed only for the first entry, generally within the first 10 min. The OFT occurred in a box-shaped apparatus measuring 36 cm × 36 cm with a black interior, open at the top. The tests were video-recorded and manually evaluated by two blinded, trained researchers.

Control group animals were gavaged with water and those of the treatment groups with AYA suspension, then immediately placed in the OFT apparatus. Behavior was evaluated in terms of (1) number of crossings, (2) latency to enter the central area of the device, (3) grooming behavior, and (4) the time spent in the central perimeter of the apparatus.

One-way ANOVA was used to compare the latency to enter the center of the apparatus. A general estimating equation—using linear-regression mixture models for repeated measures with maximum likelihood approximation—was used to compare groups across time for the number of crossings, grooming behavior, and time spent in the center of the apparatus. Pairwise Bonferroni post-hoc correction tests for multiple comparisons were used to identify the mean differences between groups across time. Results were considered significant if the *p*-value was <0.05. All analyses were performed using the 22nd version of the IBM SPSS computer package.

RESULTS AND DISCUSSION

No intergroup differences were observed in any of the assessed behavioral parameters over the first 30 min after gavage, which included the latency to enter the center of the apparatus for the first time ($F = 1.29$, $p = 0.29$). In addition, no differences were observed in grooming behavior over the 40 min observation period.

The 500 mg kg^{-1} group displayed a reduction in time spent at the center of the apparatus ($\beta = -0.546$, $p < 0.001$) and in the number of crossings ($\beta = -1.99$, $p < 0.001$) in comparison to the control group *only in the last 10 min of the observation* (mean difference = 14.65 seconds, $p = 0.006$, and mean difference 31.3, $p = 0.035$, respectively). The other experimental groups (125 mg kg^{-1} and 250 mg kg^{-1} treatments) did not differ from the control group in any period of observation (Figure 1).

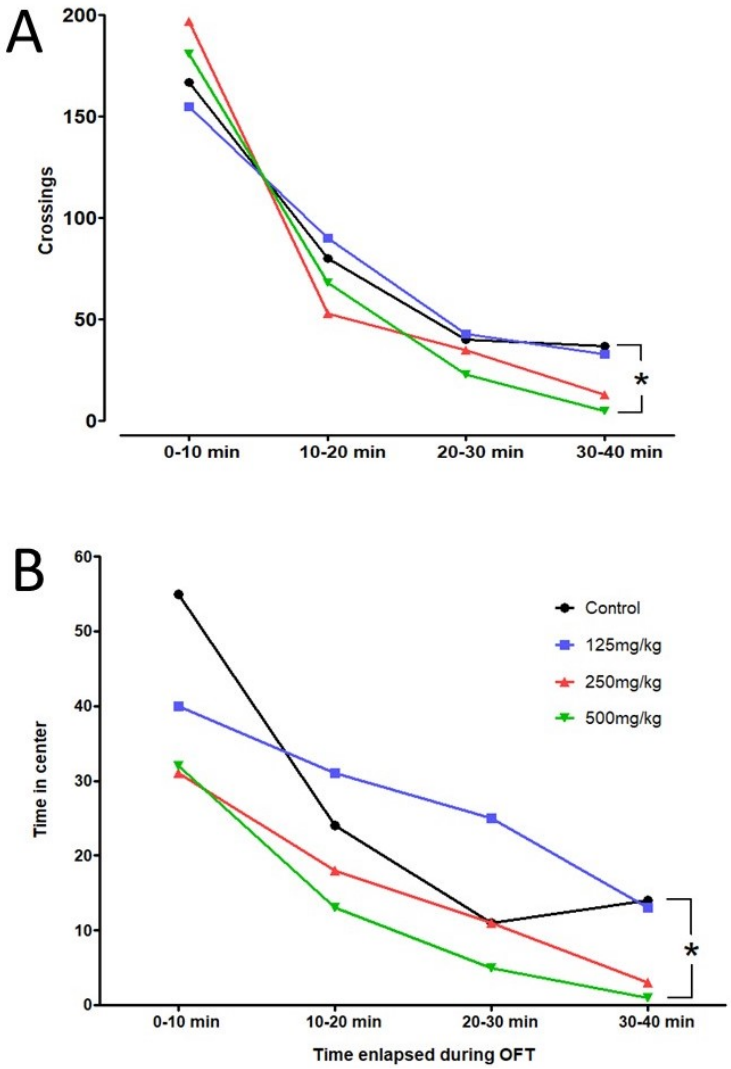

FIGURE 1 - The lines represent the number of line crossings (A) and time spent in the center of the apparatus (B) of 0 mg kg^{-1} (control; black line), 125 mg kg^{-1} (blue line), 250 mg kg^{-1} (red line), and 500 mg kg^{-1} (green line) of AYA intake over time in the OFT. Results with statistically different values ($p < 0.05$) are marked with an asterisk (*) when compared to the control group.

In general, the results indicated that AYA intake did not affect habituation in the OFT apparatus. Habituation in animal models is observed by a reduction in locomotor activity and exploratory behavior with time of exposure to the experimental apparatus. Since AYA is an acid drink (pH ≈ 3.8) with important psychoactive effects and may cause nausea and vomiting, it is possible that its intake by rats could affect habituation. Nevertheless, our results identified no behavioral modification in the rats treated with AYA in comparison to the control group in the initial 30 min of observation. In fact, a similar decrease in locomotor activity and exploratory behavior was observed in all groups over the time spent in the apparatus, which clearly indicates that the taste and contiguous effects of AYA ingestion did not interfere with the habituation of animals in the OFT.

The results also indicate that two variables must be simultaneously considered to evaluate the behavioral effects of acute AYA intake in rats subjected to the OFT paradigm: (1) the time (latency) between gavage and the onset of perceptible behavioral alterations and (2) the quantity of AYA intake (mg kg^{-1}) required to modify behavior. The group treated with the higher dose of AYA (500 mg kg^{-1}) displayed a reduction—only after 30 min—

in the time spent in the central perimeter of the apparatus and in the number of crossings, as compared with the control group. These results should be considered when planning preclinical studies in which animals are treated with acute doses of AYA. Pic-Taylor *et al*. (2015), for example, began the evaluation of high doses of AYA effects on Wistar rats only one hour after gavage, when behavioral alterations were likely to have been occurring for approximately 30 min.

These results are also consistent with those of Castro-Neto *et al*. (2013), who showed that the dosage of AYA differentially affected neurochemical outcomes in the brains of rats acutely treated with three different doses of AYA: 250 mg kg^{-1}, 500 mg kg^{-1}, and 800 mg kg^{-1}. All doses increased the rate of utilization of monoamines in the amygdala and GABA in the hippocampus; however, only the animals treated with 500 mg kg^{-1} displayed a decrease in GABA in the amygdala, and animals treated with 500 mg kg^{-1} or more showed a reduction in glycine in the amygdala (Castro-Neto *et al*., 2013; Ruffell *et al*., 2020).

Our study had several strengths and limitations. To the best of our knowledge, this is the first study that aimed to characterize the behavior of Wistar rats subjected to OFT after acute treatment with AYA. Furthermore, non-normally distributed data are quite common in behavioral research with animal models—particularly in models with repeated measures— significantly limiting the use of more traditional statistical models. In this regard, the use of generalized estimating equations to analyze repeated data has documented advantages for estimation models (Pekár, Brabec, 2018). One notable limitation is that when observations were suspended (at the 40^{th} minute), the effects of AYA were probably still present; we have therefore documented the onset—but not the suppression—of AYA behavioral effects.

Based on these results, we suggest that future behavioral investigations using AYA in the OFT—and probably in other experimental paradigms—should use AYA quantities equal to or higher than 500 mg kg^{-1} and begin observation at least 30 min after gavage. The approximate timing of the suspension of AYA behavioral effects requires further study.

CONFLICTS OF INTEREST

None.

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