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Exposure to ayahuasca induces developmental and behavioral alterations on early life stages of zebrafish

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ABSTRACT

Ayahuasca is a psychoactive concoction prepared from the plants *Banisteriopsis caapi* and *Psychotria viridis* which are used ancestrally by Amazonian Indian populations and more recently, by Christian religious groups in Brazil and other countries. The aims of the present study were to identify the effects of ayahuasca on zebrafish embryo development and neurobehavior. Toxicity and developmental endpoints for zebrafish embryos were assessed from 0 to 1000 mg/L over 96 h of exposure. The effects on locomotor activity of zebrafish larvae were assessed using a video tracking system (ZebraBox) from 0 to 20 mg/L and after 120 and 144 h of exposure. The LC_{50} of ayahuasca in zebrafish was determined as 236.3 mg/L. Ayahuasca exposure caused significant developmental anomalies in zebrafish embryos, mainly at the highest concentration tested, including hatching delay, loss of equilibrium, edema and the accumulation of red blood cells. Embryo behavior was also significantly affected, with decreased locomotor activity at the highest concentration tested. These results are in accordance with data obtained in mammal studies highlighting the possible risks of uncontrolled use of ayahuasca. Further research employing more specific behavior analysis could provide additional data on both therapeutic benefits and possible toxicological risk of ayahuasca.

1. Introduction

Ayahuasca is a hallucinogenic plant extract produced mainly by the concoction of two plants found in South America: *Psychotria viridis* and *Banisteriopsis caapi,* which contain N,N-dymethyltryptamine (DMT) and β-carbolines (harmine, harmaline and tetrahydro-harmaline), respectively. This beverage has been incorporated in Christian rituals in Brazil since the 1930s, and more recently in other South American countries, North America, Europe and Asia [1–4]. The use of ayahuasca for religious purposes is considered safe [5] and although its commercialization is currently prohibited by Brazilian legislation [6], it is possible to purchase the beverage and its constituents online and in tour packages to experience ayahuasca in the forest [7]. The association of ayahuasca with other drugs such as tricyclic and selective serotonin reuptake inhibitor (SSRI) antidepressants is potentially harmful and may lead to the development of serotonin syndrome [8].

The psychotropic action of ayahuasca involves the serotonin system and is only possible through the synergistic interactions between the main constituents of the infusion. When ingested, DMT is metabolized and inactivated by intestinal and liver enzyme monoamine oxidases (MAO) resulting in a limited effect on the nervous system. However, the β-carbolines present in ayahuasca inhibit MAO activity and prevent the inactivation of DMT, thereby allowing it to reach the brain and act as a serotonin receptor agonist [9,10]. DMT is structurally related to the endogenous neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) and has affinity for serotonin receptors, mainly the 5-HT_{2A}, what explains its psychedelic effects [5].

Several human studies have investigated the therapeutic properties of ayahuasca, predominantly to treat mood disorders [11–13]. Furthermore, the potential anti-depressive effect of ayahuasca and its

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components has been documented in animal models [14–16]. Rodent models have historically been used to investigate the neurobehavioral mechanisms of hallucinogenic drugs [17]. However, to evaluate a greater number of potential drugs it is necessary to expand the spectrum of model organisms to determine the full magnitude of both the therapeutic benefits and any associated toxicological risks [18].

In this context, zebrafish (*Danio rerio*) is rapidly emerging as a useful model to investigate hallucinogenic drug effects in neuropsychopharmacological behaviour [18–21]. Recently, behavioral tests used to model anxiety-like behaviors in rodents were successfully adapted to zebrafish including larval stages, showing to parallel rodents and clinical data [18,19]. In addition to its small size, zebrafish is easily bred in the laboratory, cost-efficient as it can be housed in large numbers in a relatively small space, and has external fertilization, producing large clutches of transparent embryos that are especially suited for morphological observation of developmental processes. The zebrafish genome has been sequenced and is well-characterized, showing high levels of structural conservation and genetic homology to humans [22]. Moreover, zebrafish possesses the classic complete neurotransmitter structure with serotonergic, opioidergic, glutamatergic, histaminergic, cholinergic and monoaminergic systems, which are also highly conserved between humans and other mammals [23–26].

The aim of the present study was to evaluate the effects of ayahuasca on zebrafish early life stages. The parameters selected comprised several levels from lethality to behavioral assessment.

2. Materials and methods

2.1. Ayahuasca infusion

The ayahuasca was provided by the *União do Vegetal* (UDV) religious group based in Brasilia, Brazil. The infusion was lyophilized (100 mL infusion yielded 0.162 g lyophilized powder), and the dry material stored at −20 °C prior to use. Quantitative analysis by GC-MS/MS determined the infusion composition as: 1.56 mg/mL harmine, 0.122 mg/mL harmaline and 0.141 mg/mL DMT (for more details, see Pic-Taylor et al. [15], and Santos et al. [27]). Tetrahydroharmine was not analyzed.

2.2. Zebrafish maintenance and embryo collection

Zebrafish embryos were provided by the facility established at the Department of Genetics and Morphology in the University of Brasilia, Brazil. Adult organisms were kept in a ZebTEC (Tecniplast, Italy) recirculating system and maintained in tanks with reverse osmosis and activated carbon filtered water. Fish were cultivated in the aquatic facility with a photoperiod cycle of 12:12 h (light:dark); water temperature of 26.0 ± 1 °C; conductivity of 750 ± 50 µS/cm; pH 7.0 ± 0.5, and dissolved oxygen ≥ 95% saturation.

Zebrafish eggs were obtained by breeding in the iSpawn breeding system (Tecniplast). Males and females (at a ratio of 1 male to 2 females) were sequentially added to system 24 h prior to breeding and segregated by a divider. The division was removed early in the morning and the spawning platform lifted to initiate spawning. Eggs were collected immediately post-mating, rinsed in water, and inspected under a stereomicroscope (SMZ 1500, Nikon Corporation). Unfertilized eggs and those with cleavage irregularities or injuries were discarded.

2.3. Zebrafish embryo toxicity assay

The assay was based on the OECD testing guideline n° 236 [28] for the fish embryo toxicity (FET) test. A total of 20 embryos per replicate were exposed in triplicate in 24-well plates (2 mL of test solution per well) to increasing concentrations of the ayahuasca infusion: 0; 0.064; 0.3; 1.6; 8; 40; 200 and 1000 mg/L of the lyophilized material diluted in zebrafish culture water. These concentrations were chosen based on a range-finding test previously carried out to derive the LC50. The test was initiated immediately after fertilization and run for 96 h. Embryos were observed daily under a stereomicroscope and the following parameters evaluated: mortality, incidence of pericardial edema and red blood cell accumulation (clutch of red blood cells), malformations, hatching, equilibrium (represented by embryos side-lying in the bottom of the microplate well) and developmental delay.

Based on the results of this assay, ayahuasca concentrations that did not induce any abnormalities or mortality were selected for the locomotor behaviour assay.

2.4. Behavioral assessment

The effects of ayahuasca on the locomotor activity of zebrafish larvae was assessed using the ZebraBox-ZEB 478 tracking system (software version 3.22, Viewpoint Life Sciences, Lyon, France) established at the Department of Biology, University of Aveiro, Portugal. The maintenance of zebrafish organisms, embryo collection and test settings were performed under similar conditions as described in *2.2*. Locomotion was evaluated for 24 embryos per treatment, including the control, in 96-well plates (one embryo per well) at 120 and 144 hpf (hours post fertilization). These time points were chosen based on previous research that showed that they have the most robust response at both light and dark periods [29]. Sublethal concentrations: 0.0064, 0.032, 0.16, 0.8, 4 and 20 mg/L were used to assess locomotor activity. The ZebraBox system monitors movement by automated video recording with an infrared camera (25 images per second). Zebrafish larvae typically show less locomotion during light periods. Therefore, movement was stimulated by applying light:dark intervals, according to Andrade et al. [30]. Briefly, embryonic movements were recorded during light-dark intervals over a period of 20 min (5 min light, 10 min dark, 5 min light). For each replicate, the distance moved in 1 min intervals was recorded. Only data from the dark period was used to calculate the differences between control and treated embryos. The calculated total distance moved refers to the total swimming distance of the larvae during each measurement period.

2.5. Statistical analyses

Ayahuasca lethal concentration (LC_{50}) and effect concentration (EC_{50}) values were calculated for the zebrafish model by fitting log-logistic dose response curves using the extension package drc for the statistical environment R [31].

A one-way analysis of variance (ANOVA) with appropriate post-hoc test (Dunnett's or Dunn's test) was conducted to potentially derive the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC), and to analyze the zebrafish larvae locomotor behavior data. The type of ANOVA (parametric or nonparametric) and post-hoc tests were chosen depending on whether normality and homoscedasticity of data were demonstrated by analysis of the residuals with the Shapiro-Wilks test.

Test statistics and normality analysis were conducted using the SigmaPlot Ver 12.5 software (SysSat, San Jose, California, USA) with a significance level of equal or below 0.05 indicating statistically significant results.

3. Results

3.1. Ayahuasca concentration-response profile of zebrafish embryos

Fertilized zebrafish eggs were exposed to different concentrations of ayahuasca infusion for 96 h. The control group presented normal embryonic development as described in Kimmel et al. [32], and mortality remained below 10% throughout the experiment, thereby satisfying the validation criteria of the OECD 236 protocol [28]. The calculated LC50 and EC50 values for zebrafish embryos exposed to ayahuasca are shown

Table 1
Estimated EC_{50} and LC_{50} values for ayahuasca after 96 h of exposure[a].

Developmental Parameter	24 h	48 h	72 h	96 h
Hatching	n.e.	n.d.	256.5 ± 10*10[6b]	–
Partial Hatching	n.e.	n.e.	n.d.	150.8 ± 85.7
Equilibrium	n.e.	n.e.	n.e.	138.4 ± 109.5#
Red blood cell accumulation	n.e.	n.e.	n.d.	223.7 ± 168.8
Edema	n.e.	n.e.	n.d.	129.2 ± 64.2
Mortality	442.1 ± 243.7	414.9 ± 221.9	414.95 ± 221.89	236.4 ± 155.8

[a] ayahuasca concentration (mg/L) ± standard errors, calculated from the adjustment of logistic regression.
[b] due to high standard error, these values are indicative only; n. e. no effect on the endpoint analysed; n. d. endpoint not determined (no effect or only effects below a 50% level); - endpoint not analysed.

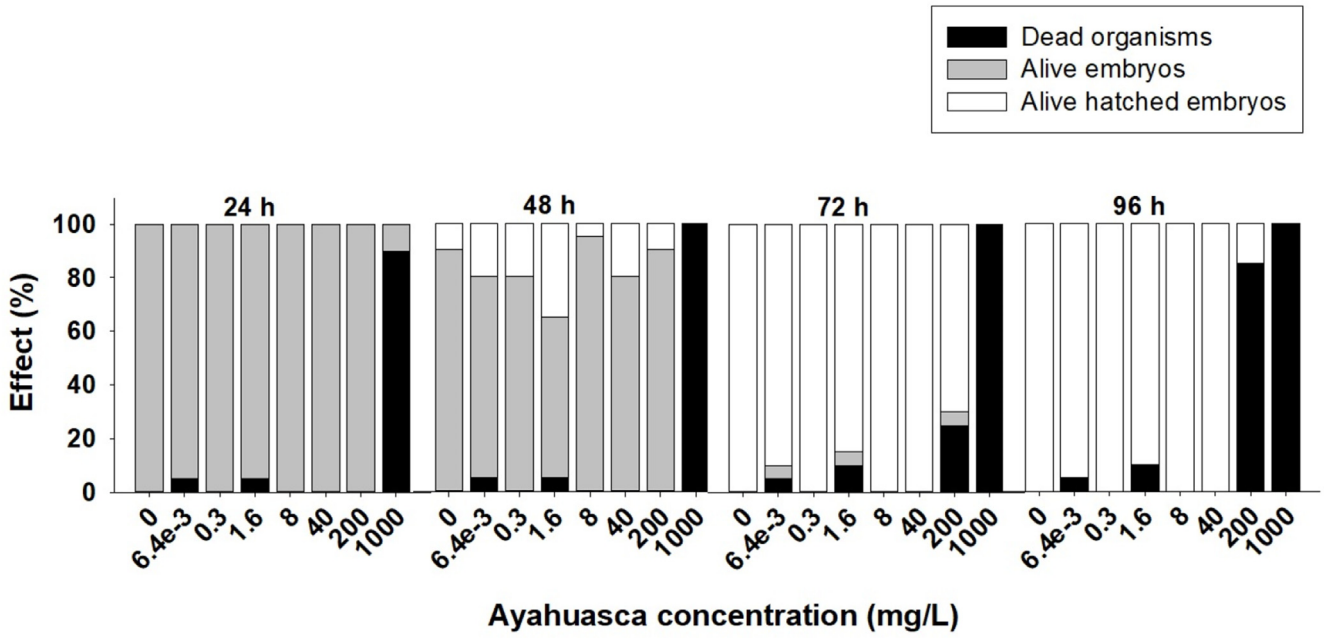

Fig. 1. Overview of ayahuasca effects on zebrafish embryo survival after 96 h of exposure.

in Table 1. A 96 h LC_{50} value of 236.3 mg/L (corresponding to 0.02 mg/mL DMT, 0.017 mg/mL harmaline and 0.22 mg/mL harmine) was determined.

Embryos exposed to 1000 mg/L showed 100% mortality after the first 48 h of exposure, as shown in Fig. 1. At 200 mg/L, mortality was only significant after hatching at 72 h of exposure, reaching more than 90% mortality at 96 hpf (Fig. 1).

Ayahuasca exposure also affected zebrafish embryo development. At 48 hpf, embryos exposed to ayahuasca concentrations ranging from 0.0064 to 40 mg/L showed precocious hatching, which was significant at 0.3 and 1.6 mg/L when compared to controls (Fig. 2a). At a higher concentration (200 mg/L), hatching was below 10%, the same rate found for the control group.

At 72 hpf, 100% of the control and over 90% exposed embryos hatched out of the chorion, except for the 200 mg/L concentration in which a significantly decreased hatching rate was observed (Fig. 2b). In addition to a decreasing hatching rate, ayahuasca also caused an increase in partial hatching (embryos presenting the tail out of the eggshell but the head remained attached to the chorion, see Fig. 2iv). Approximately 40% of the hatches occurring at 200 mg/L were partial hatches, as observed in Fig. 2c.

At 72 hpf, in addition to decreased hatching, ayahuasca affected the equilibrium of exposed embryos following a concentration-dependent pattern. However, this was only significant at 40 mg/L (see Fig. 3a). Concerning morphological effects, the most important anomalies observed were an increase in the occurrence of edema and red blood cell accumulation after 96 hpf at 200 mg/L (Fig. 3 b–c, iii and iv).

3.2. Locomotor response of zebrafish to ayahuasca

Fig. 4 shows the zebrafish embryo swimming behavior test results. At 120 hpf, a significant reduction in the total distance moved was observed at 20 mg/L, and at 144 hpf, this reduction was observed at the two highest doses tested.

4. Discussion

To the best of our knowledge, no studies have investigated the acute effects of ayahuasca on zebrafish early life stages. Other studies with classical serotonergic hallucinogens (LSD, MDMA, mescaline, ibogaine) have reported effects on physiological markers and the behavior of adult zebrafish (for a review see Neelkantan et al. [19]). In our study, the 96 h LC_{50} for zebrafish early life stages was 236.3 mg/L (corresponding to 0.02 mg/mL DMT, 0.017 mg/mL harmaline and 0.22 mg/mL harmine). Gable [33] estimated the oral LD_{50} for DMT (one of the components of ayahuasca infusion), to be 160 mg/kg bw (body weight) in mice. In another study conducted in female Wistar rats, Pic-Taylor and co-authors [15] found that the lethal oral dose of ayahuasca was greater than 50 times the ritual dose in humans, corresponding to 15.1 mg/kg bw DMT, 13.1 mg/kg bw harmaline and 167 mg/kg bw harmine.

The increased mortality observed at the highest concentrations are

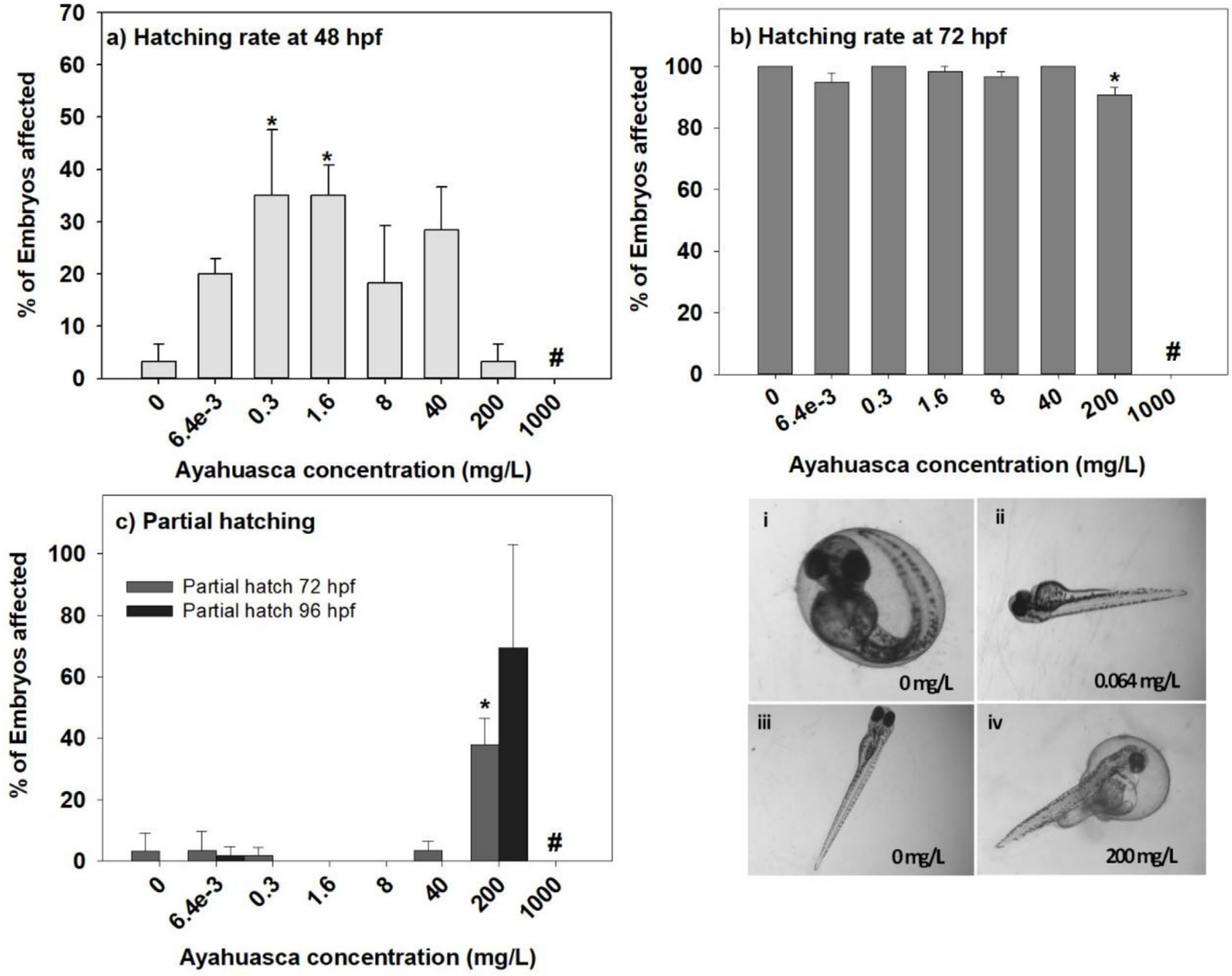

Fig. 2. Sublethal effects of ayahuasca infusion on zebrafish embryos: a) Hatching rate at 48 hpf; b) Hatching rate at 72 hpf; c) Partial hatching at 72 and 96 hpf; Zebrafish embryo phenotypes after exposure to ayahuasca: i) Control group at 48 hpf (5× amplification); ii) Hatched larva exposed to 0.064 mg/L at 48 hpf (3.2× amplification); iii) Control group at 96 hpf (3.2× amplification); iv) Partially hatched embryo exposed to 200 mg/L after 96 hpf (3.2× amplification). Asterisks correspond to significant significance ($p < 0.05$). # denotes concentrations at which 100% mortality occurred.

probably related to the serotoninergic action of DMT present in ayahuasca. Serotonergic stimulation may cause cardiovascular collapse leading to intravascular coagulation, cerebral infarct or cerebral haemorrhage ultimately causing death [1,5,8]. Our results also demonstrated that higher concentrations of ayahuasca exposure caused edema and red blood cell accumulation as discussed below. An acute study conducted in rats showed that a high dose of ayahuasca (30 X the usual dose) induced neuronal activation in specific brain regions involved in serotoninergic neurotransmission and caused some neuronal injury [15]. Likewise, pregnant rats exposed to ayahuasca for 15 days (2 to 8X the usual dose) had a significant decrease in the number of viable neurons in the same regions involved in serotoninergic neurotransmission [34]. About half of pregnant rats exposed to the higher doses (4 and 8X the usual dose) died after 2–13 days of exposure, showing signs serotoninergic syndrome. It is likely that ayahuasca affects fish embryo in the same way and higher doses would lead to death by over stimulation of serotonin regions.

Ayahuasca exposure also affected the hatchability of zebrafish embryos causing an inhibition at low and an increasing at highest concentrations. Hatching is a critical period in zebrafish embryo development, which occurs within 48–72 hpf under laboratory conditions [32]. According to Hallare et al. [35], different mechanisms may influence the hatching process of zebrafish embryos, including the activity of the embryo inside the eggshell and the distribution (failure or induction) of the hatching enzyme chorionase. Precocious hatching is probably due to an increased muscular movement that enabled the embryos to break through the outer layers of the eggshell at low concentrations. On the other hand, embryos exposed to higher ayahuasca concentrations may have caused the opposite effect, reducing the ability of the larvae to break the egg envelopes.

Along with the decrease in hatching, ayahuasca caused loss of equilibrium in exposed embryos following a concentration-dependent pattern. This effect may indicate an early signal of behavioral changes caused by ayahuasca that may affect the motor function of exposed embryos. Furthermore, ayahuasca also affected the development of embryos causing morphological alterations such as edemas and red blood cell accumulation. These results agree with a study in rats conducted by our research group using the same ayahuasca material [34]. Fetus from rats exposed to 2X the ayahuasca human ritual dose (corresponding to 6.7 mg of harmine, 0.52 mg of harmaline and 0.60 mg of DMT per kg bw/day) or higher showed soft tissue anomalies (mostly variations), including dilated cerebral ventricles, malpositioned organs

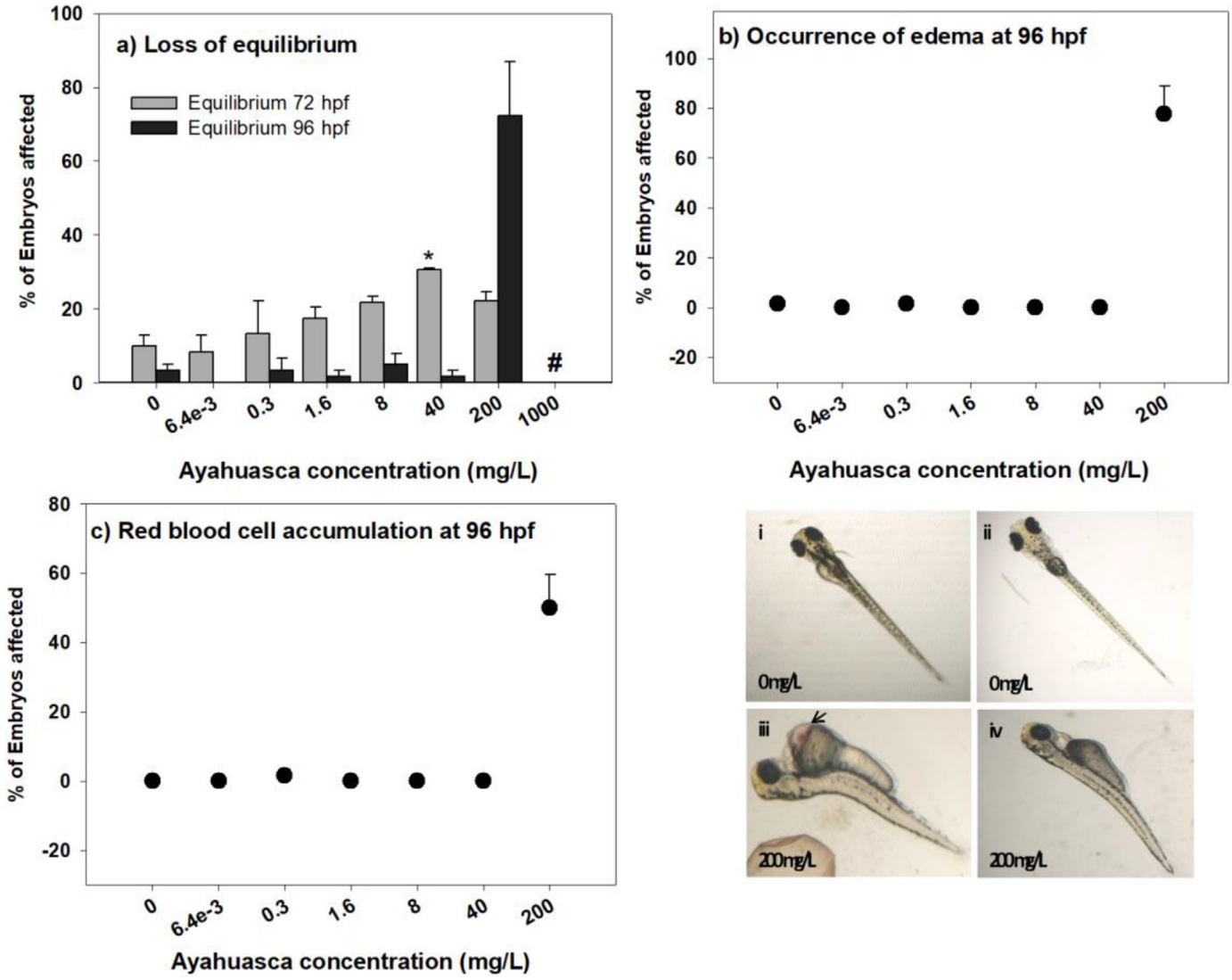

Fig. 3. Sublethal effects of ayahuasca infusion on zebrafish embryos: a) Loss of equilibrium after 72 and 96 hpf; b) Occurrence of edema after 96 hpf; c) Red blood cell accumulation after 96 hpf; Phenotypes of zebrafish embryos after exposure to ayahuasca: i) Control group at 72 hpf (3.2× amplification); ii) Control group at 96 hpf (3.2× amplification); iii) Larva exposed to 200 mg/L after 72 hpf presenting edema and red blood cell accumulation (black arrow) (5× amplification); iv) Larva exposed to 200 mg/L after 96 hpf showing edema (5× amplification). Asterisks correspond to significant significance ($p < 0.05$). # denotes concentrations at which 100% of mortality occurred. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(kidneys, testes, ovaries, and uterus), and abnormal liver shape, in addition to delayed ossification [34].

Overall, ayahuasca exposure caused a significant decrease in the locomotor activity of zebrafish larvae. Locomotor assays using zebrafish larvae have recently gained prominence in the development and screening of common drugs [18]. However, most studies were conducted in adults. Adult zebrafish exposed to typical serotonergic drugs (psychedelic mind-altering agents) such as lysergic acid diethylamide (LSD), mescaline, psilocybin, showed no alterations in the main motor activity expressed as distance travelled and velocity [36–38], although effects on anxiety-related behavior, social behavior and cognitive functions were observed. A recent study showed a concentration-dependent (1 and 3 ml/L) reduction in locomotor activity (reduced swimming speed and less distance travelled), and hypoactivity of adult zebrafish exposed to ayahuasca [39].

The ayahuasca component DMT is a serotonin receptor agonist [40]. Studies conducted in adult teleost fish determined that increased serotonin levels significantly decreased spontaneous swimming activity [41–43]. Similarly, our results demonstrated a decrease in the total swimming distance moved by the zebrafish larvae after exposure to ayahuasca which may also be related to increased serotonin in synaptic regions. Airhart et al. [44] reported a significant decrease in the swimming activity of zebrafish larvae exposed to fluoxetine, an SSRI, which was correlated with a decrease in the concentration of serotonin transporter protein (SERT) and 5-HT_{1A} transcripts in the spinal cord.

This reduction in locomotor behavior after ayahuasca exposure seems to correlate well with data obtained for rodents. A study conducted in female Wistar rats also demonstrated a significant decrease in locomotion after ayahuasca acute exposure (Pic-Taylor et al. [15]). Common behavioral screens used to model stress and anxiety in rodents were recently successfully adapted to adult and larvae of zebrafish, showing to parallel rodents and clinical data [19,20,45–47]. In addition, the powerful video-tracking tools developed in recent years, and well-defined behavioral phenotypes [48,49] make zebrafish larvae a reliable high-throughput model to study the effects of various drugs, including mixtures and/or concoctions, such as ayahuasca.

Furthermore, recent studies of a wide range of hallucinogenic drugs, including serotonergic and glutamatergic agents [36,46,50], have demonstrated that behavioral and physiological phenotypes of zebrafish are highly sensitive and appear to correlate with the data obtained in

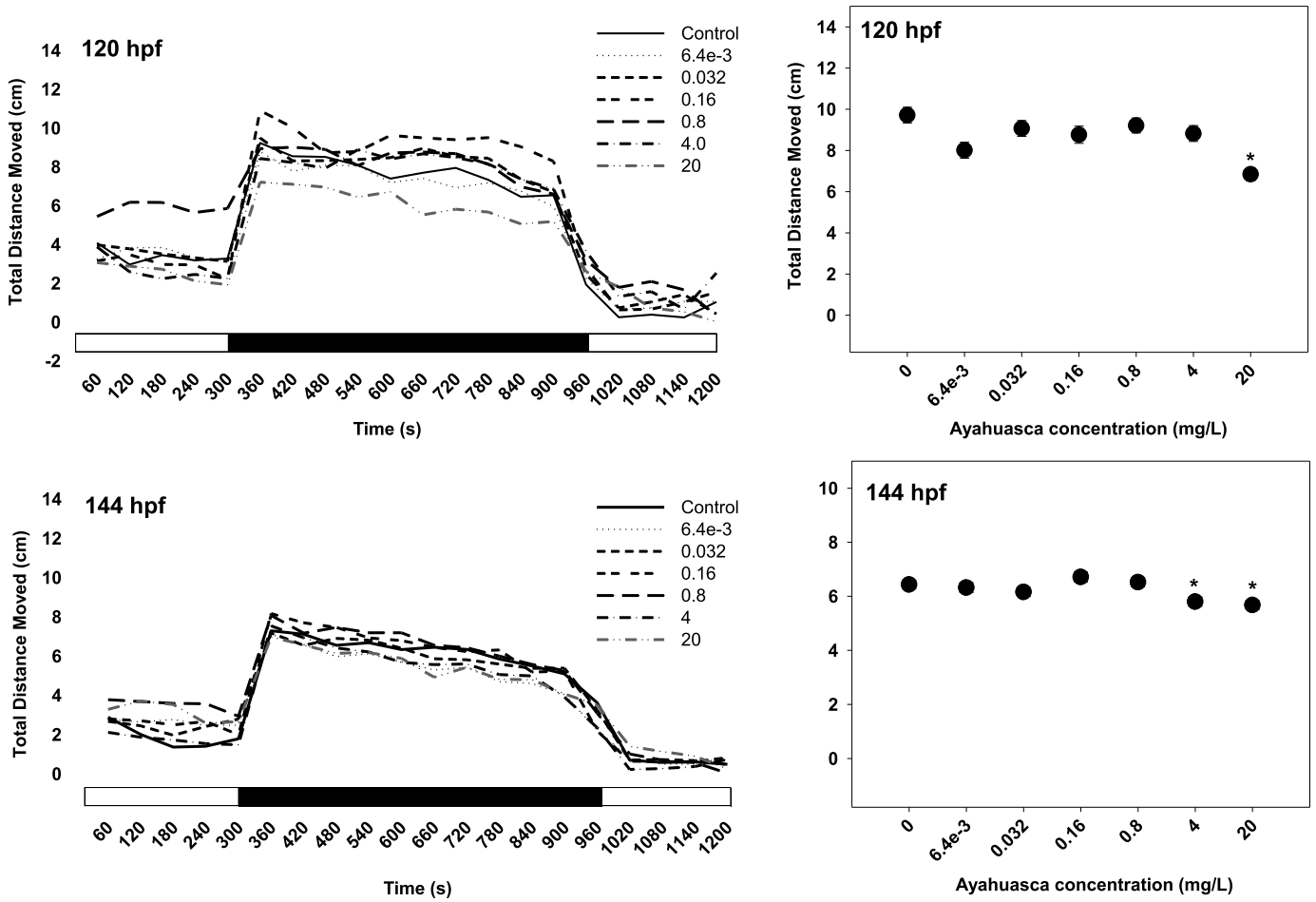

Fig. 4. Effects of ayahuasca on zebrafish swimming behavior: total distance moved after 120 and 144 hpf. Left panel: overview of zebrafish embryo behavior throughout the light and dark periods. The light and dark rectangles represent light and dark periods, respectively. Right panel: total distance moved during the dark periods only. Values are mean values ± standard error and asterisks indicate significant significance ($p < 0.05$).

mammals. Most of behavioral studies conducted to date used adult zebrafish. Therefore, there is a lack of larval zebrafish locomotor data available for the main classes of hallucinogenic drugs [19], a gap to which this paper can have an important contribution.

5. Conclusion

The effects of ayahuasca on the development and locomotor behavior of zebrafish in early life stages are reported for the first time. An LC_{50} of 236.3 mg/L was determined. Ayahuasca exposure induced several developmental anomalies such as hatching delay, loss of equilibrium, edema and red blood cell accumulation. The locomotor activity of zebrafish larvae was decreased at concentrations as low as 4 mg/L. Our results correlated well with available ayahuasca studies in rats. As referred before, zebrafish share substantial physiological and genetic homology with humans and other mammals, possessing the classic neurotransmitters structure, receptors and hormones. The use of ayahuasca under the religious context is safe [51], however caution should be taken when the beverage is use for recreation since deleterious effects are caused by high doses. Further studies of zebrafish larvae employing more specific behavior analysis could help to understand both therapeutic benefits and possible toxicological risk of ayahuasca.

Ethical standards

The experiments are in accordance with the current laws of the country in which they were performed. The study was approved by the ethics committee of the University of Brasilia (Reference No. UnB doc: 100226/2014).

Conflicts of interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cbi.2018.08.001.

Transparency document

Transparency document related to this article can be found online at

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References

[1] J.H. Halpern, Hallucinogens and dissociative agents naturally growing in the United States, Pharmacol. Ther. 102 (2004) 131–138, https://doi.org/10.1016/j.pharmthera.2004.03.003.

[2] K.W. Tupper, The globalization of ayahuasca: Harm reduction or benefit maximization? Int. J. Drug Pol. 19 (2008) 297–303, https://doi.org/10.1016/j.drugpo.2006.11.001.

[3] B.C. Labate, H. Jungaberle, The Internationalization of ayahuasca, Zurich Switzerland, Lit Verlag, 2011 978-3-643-90148-4446 pp.

[4] Health Canada, Letter of Exemption N. 17-106067-547, (June 5th, 2017) (Ottawa, Canada).

[5] E. Dominguez-Clavé, J. Soler, M. Elices, J.C. Pascual, E. Alvarez, M. de la Fuente Revenga, P. Friedlander, A. Feilding, J. Riba, Ayahuasca: pharmacology, neuroscience and therapeutic potential, Brain Res. Bull. 126 (2016) 89–101, https://doi.org/10.1016/j.brainresbull.2016.03.002.

[6] CONAD, Conselho Nacional de Políticas sobre Drogas Resolução N° 1, 25 de janeiro de, Diário Oficial da União de 26/01/2010, Brasília, DF, Brazil, section 1, (2010), p. 57.

[7] D.I. Brierley, C. Davidson, Developments in harmine pharmacology — Implications for ayahuasca use and drug-dependence treatment, Prog. Neuro Psychopharmacol. Biol. Psychiatr. 39 (2012) 263–272, https://doi.org/10.1016/j.pnpbp.2012.06.001.

[8] R.G. dos Santos, A critical evaluation of reports associating ayahuasca with life-threatening adverse reactions, J. Psychoact. Drugs 45 (2013) 179–188, https://doi.org/10.1080/02791072.2013.785846.

[9] J.C. Callaway, D.J. McKenna, C.S. Grob, G.S. Brito, L.P. Raymon, R.E. Poland, E.N. Andrade, E.O. Andrade, D.C. Mash, Pharmacokinetics of Hoasca alkaloids in healthy humans, J. Ethnopharmacol. 65 (1999) 243–256, https://doi.org/10.1016/S0378-8741(98)00168-8.

[10] E. Frecska, P. Bokor, M. Winkelman, The therapeutic potentials of ayahuasca: possible effects against various diseases of civilization, Front. Pharmacol. 7 (2016) 35, https://doi.org/10.3389/fphar.2016.00035.

[11] R.G. Santos, J. Landeira-Fernandez, R.J. Strassman, V. Motta, A.P.M. Cruz, Effects of ayahuasca on psychometric measures of anxiety, panic-like and hopelessness in Santo Daime members, J. Ethnopharmacol. 112 (2007) 507–513, https://doi.org/10.1016/j.jep.2007.04.012.

[12] F. de L. Osório, R.F. Sanches, L.R. Macedo, R.G. dos Santos, J.P. Maia-de-Oliveira, L. Wichert-Ana, D.B. de Araujo, J. Riba, J.A. Crippa, J.E. Hallak, Antidepressant effects of a single dose of ayahuasca in patients with recurrent depression: a preliminary report, Rev. Bras. Psiquiatr. 37 (2015) 13–20, https://doi.org/10.1590/1516-4446-2014-1496.

[13] R.F. Sanches, F. de Lima Osório, R.G. Dos Santos, L.R.H. Macedo, J.P. Maia-de-Oliveira, L. Wichert-Ana, D.B. de Araujo, J. Riba, J.A.S. Crippa, J.E.C. Hallak, Antidepressant effects of a single dose of ayahuasca in patients with recurrent depression: a SPECT study, J. Clin. Psychopharmacol. 36 (2016) 77–81, https://doi.org/10.1097/JCP.0000000000000436.

[14] D. Farzin, N. Mansouri, Antidepressant-like effect of harmane and other β-carbolines in the mouse forced swim test, Eur. Neuropsychopharmacol 16 (2006) 324–328, https://doi.org/10.1016/j.euroneuro.2005.08.005.

[15] A. Pic-Taylor, L.G. da Motta, J.A. de Morais, W.M. Junior, A. de F.A. Santos, L.A. Campos, M.R. Mortari, M.V. von Zuben, E.D. Caldas, Behavioural and neurotoxic effects of ayahuasca infusion (Banisteriopsis caapi and Psychotria viridis) in female Wistar rat, Behav. Process. 118 (2015) 102–110, https://doi.org/10.1016/j.beproc.2015.05.004.

[16] J.J. Fortunato, G.Z. Réus, T.R. Kirsch, R.B. Stringari, L. Stertz, F. Kapczinski, J.P. Pinto, J.E. Hallak, A.W. Zuardi, J.A. Crippa, J. Quevedo, Acute harmine administration induces antidepressive-like effects and increases BDNF levels in the rat hippocampus, Prog. Neuro Psychopharmacol. Biol. Psychiatr. 33 (2009) 1425–1430, https://doi.org/10.1016/j.pnpbp.2009.07.021.

[17] J.B. Hanks, J. González-Maeso, Animal models of serotonergic psychedelics, ACS Chem. Neurosci. 4 (2013) 33–42, https://doi.org/10.1021/cn300138m.

[18] A.V. Kalueff, D.J. Echevarria, S. Homechaudhuri, A.M. Stewart, A.D. Collier, A.A. Kaluyeva, S. Li, Y. Liu, P. Chen, J.J. Wang, L. Yang, A. Mitra, S. Pal, A. Chaudhuri, A. Roy, M. Biswas, D. Roy, A. Podder, M.K. Poudel, D.P. Katare, R.J. Mani, E.J. Kyzar, S. Gaikwad, M. Nguyen, C. Song, Zebrafish neurobehavioral phenomics for aquatic neuropharmacology and toxicology research, Aquat. Toxicol. 170 (2016) 297–309, https://doi.org/10.1016/j.aquatox.2015.08.007.

[19] N. Neelkantan, A. Mikhaylova, A.M. Stewart, R. Arnold, V. Gjeloshi, D. Kondaveeti, M.K. Poudel, A.V. Kalueff, Perspectives on zebrafish models of hallucinogenic drugs and related psychotropic compounds, ACS Chem. Neurosci. 4 (2013) 1137–1150, https://doi.org/10.1021/cn400090q.

[20] A.V. Kalueff, A.M. Stewart, R. Gerlai, Zebrafish as an emerging model for studying complex brain disorders, Trends Pharmacol. Sci. 35 (2014) 63–75, https://doi.org/10.1016/j.tips.2013.12.002.

[21] E.J. Kyzar, A.V. Kalueff, Exploring hallucinogen pharmacology and psychedelic medicine with zebrafish models, Zebrafish 13 (2016) 379–390, https://doi.org/10.1089/zeb.2016.1251.

[22] K. Howe, M.D. Clark, C.F. Torroja, J. Torrance, C. Berthelot, M. Muffato, J.E.J.E. Collins, S. Humphray, K. McLaren, L. Matthews, S. McLaren, I. Sealy, M. Caccamo, C. Churcher, C. Scott, J.C. Barrett, R. Koch, G.-J.J. Rauch, S. White, W. Chow, B. Kilian, L.T. Quintais, J. a Guerra-Assunção, Y. Zhou, Y. Gu, J. Yen, J.-H.H. Vogel, T. Eyre, S. Redmond, R. Banerjee, J. Chi, B. Fu, E. Langley, S.F. Maguire, G.K. Laird, D. Lloyd, E. Kenyon, S. Donaldson, H. Sehra, J. Almeida-King, J. Loveland, S. Trevanion, M. Jones, M. Quail, D. Willey, A. Hunt, J. Burton, S. Sims, K. McLay, B. Plumb, J. Davis, C. Clee, K. Oliver, R. Clark, C. Riddle, D. Eliott, G. Threadgold, G. Harden, D. Ware, B. Mortimer, G. Kerry, P. Heath, B. Phillimore, A. Tracey, N. Corby, M. Dunn, C. Johnson, J. Wood, S. Clark, S. Pelan, G. Griffiths, M. Smith, R. Glithero, P. Howden, N. Barker, C. Stevens, J. Harley, K. Holt, G. Panagiotidis, J. Lovell, H. Beasley, C. Henderson, D. Gordon, K. Auger, D. Wright, J.E.J.E. Collins, C. Raisen, L. Dyer, K. Leung, L. Robertson, K. Ambridge, D. Leongamornlert, S. McGuire, R. Gilderthorp, C. Griffiths, D. Manthravadi, S. Nichol, G. Barker, S. Whitehead, M. Kay, J. Brown, C. Murnane, E. Gray, M. Humphries, N. Sycamore, D. Barker, D. Saunders, J. Wallis, A. Babbage, S. Hammond, M. Mashreghi-Mohammadi, L. Barr, S. Martin, P. Wray, A. Ellington, N. Matthews, M. Ellwood, R. Woodmansey, G. Clark, J.D. Cooper, A. Tromans, D. Grafham, C. Skuce, R. Pandian, R. Andrews, E. Harrison, A. Kimberley, J. Garnett, N. Fosker, R. Hall, P. Garner, D. Kelly, C. Bird, S. Palmer, I. Gehring, A. Berger, C.M. Dooley, Z. Ersan-Ürün, C. Eser, H. Geiger, M. Geisler, L. Karotki, A. Kirn, J. Konantz, M. Konantz, M. Oberländer, S. Rudolph-Geiger, M. Teucke, K. Osoegawa, B. Zhu, S.A. Rapp, C. Widaa, F. Langford, N.P. Yang, J. Carter, Z. Harrow, J. Ning, S.M.J. Herrero, A. Searle, R. Enright, R.H.A. Geisler, C. Plasterk, M. Lee, P.J. Westerfield, de Jong, L.I. Zon, J.H. Postlethwait, C. Nüsslein-Volhard, T.J.P. Hubbard, H. Roest Crollius, J. Rogers, D.L. Stemple, D. Elliot, D. Eliott, G. Threadgold, G. Harden, D. Ware, S. Begum, B. Mortimore, B. Mortimer, G. Kerry, P. Heath, B. Phillimore, A. Tracey, N. Corby, M. Dunn, C. Johnson, J. Wood, S. Clark, S. Pelan, G. Griffiths, M. Smith, R. Glithero, P. Howden, N. Barker, C. Lloyd, C. Stevens, J. Harley, K. Holt, G. Panagiotidis, J. Lovell, H. Beasley, C. Henderson, D. Gordon, K. Auger, D. Wright, J.E.J.E. Collins, C. Raisen, L. Dyer, K. Leung, L. Robertson, K. Ambridge, D. Leongamornlert, S. McGuire, R. Gilderthorp, C. Griffiths, D. Manthravadi, S. Nichol, G. Barker, S. Whitehead, M. Kay, J. Brown, C. Murnane, E. Gray, M. Humphries, N. Sycamore, D. Barker, D. Saunders, J. Wallis, A. Babbage, S. Hammond, M. Mashreghi-Mohammadi, L. Barr, S. Martin, P. Wray, A. Ellington, N. Matthews, M. Ellwood, R. Woodmansey, G. Clark, J.D. Cooper, J.D. Cooper, A. Tromans, D. Grafham, C. Skuce, R. Pandian, R. Andrews, E. Harrison, A. Kimberley, J. Garnett, N. Fosker, R. Hall, P. Garner, D. Kelly, C. Bird, S. Palmer, I. Gehring, A. Berger, C.M. Dooley, Z. Ersan-Ürün, C. Eser, H. Geiger, M. Geisler, L. Karotki, A. Kirn, J. Konantz, M. Konantz, M. Oberländer, S. Rudolph-Geiger, M. Teucke, C. Lanz, G. Raddatz, K. Osoegawa, B. Zhu, A. Rapp, S. Widaa, C. Langford, F. Yang, S.C. Schuster, N.P. Carter, J. Harrow, Z. Ning, J. Herrero, S.M.J. Searle, A. Enright, R. Geisler, R.H.A. Plasterk, C. Lee, M. Westerfield, P.J. de Jong, L.I. Zon, J.H. Postlethwait, C. Nüsslein-Volhard, T.J.P. Hubbard, H. Roest Crollius, J. Rogers, D.L. Stemple, The zebrafish reference genome sequence and its relationship to the human genome, Nature 496 (2013) 498–503, https://doi.org/10.1038/nature12111.

[23] J. Kaslin, P. Panula, Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (Danio rerio), J. Comp. Neurol. 440 (2001) 342–377, https://doi.org/10.1002/cne.1390.

[24] P. Panula, V. Sallinen, M. Sundvik, J. Kolehmainen, V. Torkko, A. Tiittula, M. Moshnyakov, P. Podlasz, Modulatory neurotransmitter systems and behavior: towards zebrafish models of neurodegenerative diseases, Zebrafish 3 (2006) 235–247, https://doi.org/10.1089/zeb.2006.3.235.

[25] C. Maximino, M.G. Lima, J. Araujo, K.R.M. Oliveira, A.M. Herculano, A.M. Stewart, E.J. Kyzar, J. Cachat, A. V Kalueff, The serotonergic system of zebrafish: genomics, neuroanatomy and neuropharmacology, Serotonin Biosynthesis, Regul. Heal. Implic (2013) 53–67.

[26] K. Yamamoto, J.O. Ruuskanen, M.F. Wullimann, P. Vernier, Differential expression of dopaminergic cell markers in the adult zebrafish forebrain, J. Comp. Neurol. 519 (2011) 576–598, https://doi.org/10.1002/cne.22535.

[27] A. de F.A. Santos, A. Luiza, S. Vieira, A. Pic-Taylor, E. Dutra Caldas, ARTICLE IN PRESS G Model Reproductive effects of the psychoactive beverage ayahuasca in male Wistar rats after chronic exposure, Rev. Bras. Farmacogn (2017) 353–360, https://doi.org/10.1016/j.bjp.2017.01.006 xxx.

[28] OECD, Test No. 236: Fish Embryo Acute Toxicity (FET) Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, 2013, https://doi.org/10.1787/9789264203709-en.

[29] S. Padilla, D.L. Hunter, B. Padnos, S. Frady, R.C. MacPhail, Assessing locomotor activity in larval zebrafish: influence of extrinsic and intrinsic variables, Neurotoxicol. Teratol. 33 (2011) 624–630, https://doi.org/10.1016/j.ntt.2011.08.005.

[30] T.S. Andrade, J.F. Henriques, A.R. Almeida, A.L. Machado, O. Koba, P.T. Giang, A.M.V.M. Soares, I. Domingues, Carbendazim exposure induces developmental, biochemical and behavioural disturbance in zebrafish embryos, Aquat. Toxicol. 170 (2016) 390–399, https://doi.org/10.1016/j.aquatox.2015.11.017.

[31] R Core Team, R: a Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2014.

[32] C. Kimmel, W. Ballard, Stages of embryonic development of the zebrafish, Dev. Dynam. 203 (1995) 253–310, https://doi.org/10.1002/aja.1002030302.

[33] R.S. Gable, Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids, Addiction 102 (2007) 24–34, https://doi.org/10.1111/j.1360-0443.2006.01652.x.

[34] L.G. da Motta, J.A. de Morais, A.C.A.M. Tavares, L.M.S. Vianna, M.R. Mortari, R.F.B. Amorim, R.R. Carvalho, F.J.R. Paumgartten, A. Pic-Taylor, E.D. Caldas, Maternal and developmental toxicity of the hallucinogenic plant-based beverage ayahuasca in rats, Reprod. Toxicol. 77 (2018) 143–153, https://doi.org/10.1016/j.reprotox.2018.03.002.

[35] a. V. Hallare, M. Schirling, T. Luckenbach, H.-R. Köhler, R. Triebskorn, Combined effects of temperature and cadmium on developmental parameters and biomarker responses in zebrafish (Danio rerio) embryos, J. Therm. Biol. 30 (2005) 7–17, https://doi.org/10.1016/j.jtherbio.2004.06.002.

[36] L. Grossman, E. Utterback, A. Stewart, S. Gaikwad, K.M. Chung, C. Suciu, K. Wong, M. Elegante, S. Elkhayat, J. Tan, T. Gilder, N. Wu, J. DiLeo, J. Cachat, A.V. Kalueff, Characterization of behavioral and endocrine effects of LSD on zebrafish, Behav. Brain Res. 214 (2010) 277–284, https://doi.org/10.1016/j.bbr.2010.05.039.
[37] A.V. Kalueff, A.M. Stewart, Zebrafish Protocols for Neurobehavioral Research, (2012).
[38] E.J. Kyzar, C. Collins, S. Gaikwad, J. Green, A. Roth, L. Monnig, M. El-Ounsi, A. Davis, A. Freeman, N. Capezio, A.M. Stewart, A. V Kalueff, Effects of hallucinogenic agents mescaline and phencyclidine on zebrafish behavior and physiology, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 37 (2012) 194–202, https://doi.org/10.1016/j.pnpbp.2012.01.003.
[39] R. Savoldi, D. Polari, J. Pinheiro-da-Silva, P.F. Silva, B. Lobao-Soares, M. Yonamine, F. Freire, A.C. Luchiari, Behavioral changes over time following ayahuasca exposure in zebrafish, Front. Behav. Neurosci. 11 (2017) 139, https://doi.org/10.3389/FNBEH.2017.00139.
[40] A.L. Halberstadt, M.A. Geyer, Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens, Neuropharmacology 61 (2011) 364–381, https://doi.org/10.1016/j.neuropharm.2011.01.017.
[41] H.A.N. Perreault, K. Semsar, J. Godwin, Fluoxetine treatment decreases territorial aggression in a coral reef fish, Physiol. Behav. 79 (2003) 719–724, https://doi.org/10.1016/S0031-9384(03)00211-7.
[42] M.J. Airhart, B. Miller, B. Turner, D. Lee, R.G. Skalko, Adverse effects of paroxetine (Paxil™) on motor development in zebrafish, Birth Defects Res. Part A Clin. Mol. Teratol. (2004) 305.
[43] K. Semsar, H.A.N. Perreault, J. Godwin, Fluoxetine-treated male wrasses exhibit low AVT expression, Brain Res. 1029 (2004) 141–147, https://doi.org/10.1016/j.brainres.2004.09.030.
[44] M.J. Airhart, D.H. Lee, T.D. Wilson, B.E. Miller, M.N. Miller, R.G. Skalko, Movement disorders and neurochemical changes in zebrafish larvae after bath exposure to fluoxetine (PROZAC), Neurotoxicol. Teratol. 29 (2007) 652–664, https://doi.org/10.1016/j.ntt.2007.07.005.
[45] C. Maximino, T.M. de Brito, A.W. da Silva Batista, A.M. Herculano, S. Morato, A. Gouveia, Measuring anxiety in zebrafish: a critical review, Behav. Brain Res. 214 (2010) 157–171, https://doi.org/10.1016/j.bbr.2010.05.031.
[46] A. Stewart, N. Wu, J. Cachat, P. Hart, S. Gaikwad, Pharmacological modulation of anxiety-like phenotypes in adult zebrafish behavioral models, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35 (2011) 1421–1431, https://doi.org/10.1016/j.pnpbp.2010.11.035.
[47] L.M. Félix, L.M. Antunes, A.M. Coimbra, A.M. Valentim, Behavioral alterations of zebrafish larvae after early embryonic exposure to ketamine, Psychopharmacology (Berlin) 234 (2017) 549–558, https://doi.org/10.1007/s00213-016-4491-7.
[48] A. V Kalueff, M. Gebhardt, A.M. Stewart, J.M. Cachat, M. Brimmer, J.S. Chawla, C. Craddock, E.J. Kyzar, A. Roth, S. Landsman, S. Gaikwad, K. Robinson, E. Baatrup, K. Tierney, A. Shamchuk, W. Norton, N. Miller, T. Nicolson, O. Braubach, C.P. Gilman, J. Pittman, D.B. Rosemberg, R. Gerlai, D. Echevarria, E. Lamb, S.C.F. Neuhauss, W. Weng, L. Bally-Cuif, H. Schneider, Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond, Zebrafish 10 (2013) 70–86, https://doi.org/10.1089/zeb.2012.0861.
[49] A.M. Stewart, J. Cachat, S. Gaikwad, K.S.L. Robinson, M. Gebhardt, A.V. Kalueff, Perspectives on experimental models of serotonin syndrome in zebrafish, Neurochem. Int. 62 (2013) 893–902, https://doi.org/10.1016/j.neuint.2013.02.018.
[50] S.M. Zakhary, D. Ayubcha, F. Ansari, K. Kamran, M. Karim, J.R. Leheste, J.M. Horowitz, G. Torres, A behavioral and molecular analysis of ketamine in zebrafish, Synapse 65 (2011) 160–167, https://doi.org/10.1002/syn.20830.
[51] P.C. Barbosa, R.J. Strassman, D.X. da Silveira, K. Areco, R. Hoy, J. Pommy, R. Thoma, M. Bogenschutz, Psychological and neuropsychological assessment of regular hoasca users, Compr. Psychiatr. 71 (2016) 95–105, https://doi.org/10.1016/j.comppsych.2016.09.003.