Behavioural Brain Research 236 (2013) 258–269

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Unique and potent effects of acute ibogaine on zebrafish: The developing utility of novel aquatic models for hallucinogenic drug research

Jonathan Cachat[a,b], Evan J. Kyzar[a,b], Christopher Collins[a], Siddharth Gaikwad[a], Jeremy Green[a], Andrew Roth[a], Mohamed El-Ounsi[a], Ari Davis[a], Mimi Pham[a], Samuel Landsman[a], Adam Michael Stewart[a,b], Allan V. Kalueff[a,b,c,*]

[a] Department of Pharmacology and Neuroscience Program, Tulane University Medical School, 1430 Tulane Avenue, New Orleans, LA 70112, USA
[b] Zebrafish Neuroscience Research Consortium (ZNRC), Tulane University Medical School, 1430 Tulane Avenue, New Orleans, LA 70112, USA
[c] ZENEREI Institute, 309 Palmer Court, Slidell, LA 70458, USA

HIGHLIGHTS

- Ibogaine is a potent hallucinogenic drug with multiple psychoactive effects.
- Ibogaine exerted robust anxiolytic-like effects on zebrafish behavior.
- Ibogane altered shoaling and coloration, but not cortisol or *c-fos* expression.
- Our results support the utility of zebrafish for hallucinogenic drug research.

ARTICLE INFO

Article history:
Received 30 May 2012
Received in revised form 23 August 2012
Accepted 28 August 2012
Available online 4 September 2012

Keywords:
Ibogaine
Hallucinogenic drugs
Zebrafish
Novel tank test
3D Reconstructions of locomotion

ABSTRACT

An indole alkaloid, ibogaine is the principal psychoactive component of the iboga plant, used by indigenous peoples in West Africa for centuries. Modulating multiple neurotransmitter systems, the drug is a potent hallucinogen in humans, although its psychotropic effects remain poorly understood. Expanding the range of model species is an important strategy for translational neuroscience research. Here we exposed adult zebrafish (*Danio rerio*) to 10 and 20 mg/L of ibogaine, testing them in the novel tank, light–dark box, open field, mirror stimulation, social preference and shoaling tests. In the novel tank test, the zebrafish natural diving response (geotaxis) was reversed by ibogaine, inducing initial top swimming followed by bottom dwelling. Ibogaine also attenuated the innate preference for dark environments (scototaxis) in the light–dark box test. While it did not exert overt locomotor or thigmotaxic responses in the open field test, the drug altered spatiotemporal exploration of novel environment, inducing clear preference of some areas over others. Ibogaine also promoted 'mirror' exploration in the mirror stimulation test, disrupted group cohesion in the shoaling test, and evoked strong coloration responses due to melanophore aggregation, but did not alter brain *c-fos* expression or whole-body cortisol levels. Overall, our results support the complex pharmacological profile of ibogaine and its high sensitivity in zebrafish models, dose-dependently affecting multiple behavioral domains. While future investigations in zebrafish may help elucidate the mechanisms underlying these unique behavioral effects, our study strongly supports the developing utility of aquatic models in hallucinogenic drug research. High sensitivity of three-dimensional phenotyping approaches applied here to behavioral effects of ibogaine in zebrafish provides further evidence of how 3D reconstructions of zebrafish swimming paths may be useful for high-throughput pharmacological screening.

1. Introduction

Ibogaine is an indole alkaloid derivative with psychoactive properties, which can be isolated from the African shrub *Tabernanthe iboga* [1,2]. At different doses, it has been used by native Western Africans as a stimulant, appetite suppressant and aid in religious ceremonies [3]. In addition to potent hallucinogenic effects [4], ibogaine is effective in the treatment of addiction to

* Corresponding author at: Department of Pharmacology, Room SL-83, Tulane University Medical School, 1430 Tulane Ave., New Orleans, LA 70112, USA.
Tel.: +1 504 988 3354; fax: +1 504 988 3354.
E-mail address: avkalueff@gmail.com (A.V. Kalueff).

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2012.08.041

opiates, methamphetamine, cocaine, and some other drugs [3,5]. Despite these potential therapeutic applications, the mechanisms of ibogaine action remain poorly understood. The pharmacological profile of ibogaine is very complex and involves multiple neuromediator systems. Structurally resembling serotonin (5-HT), ibogaine inhibits serotonin and dopamine transporters [1] and activates serotonin- (e.g., 5-HT_{2a}, 5-HT_{2c}) [6,7], opioid- (mu and kappa) [8,9] and sigma- (1 and 2) receptors [4,10,11]. The drug also acts as an antagonist of glutamate NMDA receptors [11–13], and a weak inhibitor of cholinergic muscarinic and nicotinic receptors [14]; see [15] for details of ibogaine receptorome.

While ibogaine is a controlled substance in various countries, including the United States (Schedule I), the drug does not appear to be commonly abused and is administered in medical settings in South Africa and Mexico [2]. In humans, ibogaine produces intense dream-like hallucinations which subjectively differ from those caused by classic serotonergic psychedelics [2,16] and include a vivid 'visual' phase followed by a longer 'introspective' phase [2,16]. Ibogaine can occasionally cause acute psychoses [17], whereas its anti-addictive properties have also been reported in the literature [5,18], including lasting anti-craving effects after a single ibogaine dose [19,20]. Further supporting the complex nature of ibogaine action are the prolonged effects of ibogaine in attenuating addiction and depressive symptoms [3].

Although ibogaine has been previously tested in rodent models, its effects on various animal phenotypes remain poorly understood. For example, it enhances nociception and other opioidergic effects in rodents [21] and reduces locomotion and central activity in novel environments [22]. In the plus-maze test, acute ibogaine decreases aversion to the open arms, interpreted as anxiolysis [23], while other studies reported anxiogenic-like responses [24]. Rats trained to discriminate ibogaine from vehicle did not respond to other serotonergic hallucinogens, such as lysergic acid diethylamide (LSD) and 3,4-methylenedioxymethamphetamine (MDMA) [25]. In addition to direct effects on multiple receptors, ibogaine modulates several molecular pathways, up-regulating the expression of glial cell line-derived neurotropic factor [26] and early proto-oncogenes *egr-1* and *c-fos* in the brain [27]. The development of novel high-throughput models and expanding the range of model species are important strategic directions in biological psychiatry [28], particularly useful to tackle complex effects of psychotropic drugs. Recently, there has been a remarkable resurgence of interest in hallucinogenic drugs, focusing on the mechanisms of their action in various species, as well as side effects and potential clinical applications [16,28–34]. Zebrafish (*Danio rerio*) possess high physiological similarity to humans [35–41], robust behavioral responses and a fully characterized genome [42,43], and are emerging as a sensitive and promising model for the investigation of hallucinogen-evoked states. Recent studies have reported the effects of LSD [44], MDMA [45], mescaline, phencyclidine (PCP) [46], ketamine [47,48] and salvinorin A [49,50] in adult zebrafish, emphasizing the role of specific receptor systems in the observed hallucinogenic-like phenotypes.

The pharmacological profile of ibogaine includes receptor targets that are shared with serotonergic psychedelic hallucinogens (e.g., LSD, mescaline, psilocybin), dissociative glutamatergic hallucinogens (e.g., ketamine) and hallucinogenic drugs acting via opioidergic systems (e.g., salvinorin A) [4,10,13]. The unique aspect of ibogaine action is that it affects all these targets simultaneously, most likely resulting in a complex profile that may theoretically include the actions of LSD, mescaline, psilocybin, MDMA, ketamine, PCP and salvinorin A combined. The sensitivity of zebrafish to all these drugs (see above) renders them a potentially useful experimental model to further elucidate the profile of acute ibogaine exposure. The present study aimed to evaluate the potential effects of ibogaine in several behavioral paradigms in adult zebrafish. In addition to behavioral markers, selected physiological biomarkers, validated in previous zebrafish studies, including *c-fos* gene expression (as a measure of neuronal activation [51]), and cortisol levels (as a measure of the neuroendocrine axis activation [52–54]), were examined following ibogaine treatment.

2. Methods

2.1. Animals and housing

A total of 500 adult (5–8-month-old) "wild type" short fin zebrafish (~50:50 male:female ratio) were obtained from a commercial distributor (50 Fathoms, Metairie, LA). All fish were given at least 14 days to acclimate to the laboratory environment and housed in groups of 20–30 fish per 40-L tank. Tanks were filled with filtered system water and maintained at 25–27 °C. Illumination (1000–1100 lx) was provided by ceiling-mounted fluorescent lights on a 12-h cycle (on: 6.00 h, off: 18.00 h) according to the standards of zebrafish care [55]. All fish used in this study were experimentally naïve and fed Tetramin Tropical Flakes (Tetra USA, Blacksburg, VA) twice a day. Following behavioral testing, the animals were euthanized in 500 mg/L Tricaine (Sigma–Aldrich, St. Louis, MO) and dissected on ice for further analysis. Animal experimentation in this study fully adhered to national and institutional guidelines and regulations.

2.2. Behavioral testing

Behavioral testing was performed between 11:00 and 15:00 h using tanks with water adjusted to the holding room temperature. The present study used several different behavioral tests, including the novel tank, open field (OFT), social preference, shoaling and mirror stimulation tests, as described in [44,56]. To avoid the test battery effect, each test was performed on a separate cohort of naïve fish. Prior to testing, fish were pre-exposed in a 1-L plastic beaker for 20 min to either drug-treated or drug-free vehicle solution (0.1% dimethyl sulfoxide DMSO, commonly used in zebrafish behavioral assays [46]). During testing, zebrafish behavior was recorded by 2–3 trained observers blind to the treatments, who manually scored different behavioral endpoints (inter- and intra-rater reliability in all experiments >0.85) with subsequent automated analysis of generated traces by Ethovision XT7 software (Noldus IT, Wageningen, Netherlands).

The novel tank test, used to assess zebrafish anxiety and locomotion [45,57–59], was a 1.5-L trapezoidal tank (15 cm height × 28 cm top × 23 cm bottom × 7 cm width; Aquatic Habitats, Apopka, FL) maximally filled with water and divided into two equal virtual horizontal portions by a line marking the outside walls (Fig. 1). In Experiment 1, fish were individually pre-exposed to ibogaine (10 or 20 mg/L) for 20 min (see details further), and tested in the standard 6-min novel tank test. Zebrafish behavior was recorded by trained observers, scoring the latency to reach the top half of the tank (s), time spent in top (s), number of transitions to top, as well as the number and duration (s) of freezing bouts. Freezing was defined as a total absence of movement, except for the gills and eyes, for >2 s. Trials were also recorded to a computer using a USB webcam (2.0-megapixel, Gigaware, UK) and subsequently analyzed by Ethovision XT7, assessing distance traveled (m), velocity (m/s), and meandering [44]. Ethograms in this test were also constructed by manually scoring episodes of bottom swimming, top swimming, bottom freezing and erratic movements, in order to visualize the occurrence of behaviors and the transitions between them, with the diameter of each circle reflecting the frequency of the behavioral activity, and the width and direction of each arrow representing the frequency of transitions between behaviors [44].

The light–dark test (Experiment 2), based on the natural preference of zebrafish for dark environments [60,61], was a rectangular tank (15 cm height × 30 cm length × 16 cm width) filled with water to a height of 12 cm, and divided into two equal vertical portions, demarcated by black and white coloration (Fig. 2A) [61]. Fish (n = 13 in each group) were individually introduced into the black half (facing the wall), for 5 min, and manually scored for the latency to enter(s), time spent (s), average entry duration (s), and the number of entries to the white half (due to the dark background, zebrafish behavior in the black compartment was not assessed here).

The OFT (Experiment 3) consisted of a white plastic cylinder (21 cm diameter, 24 cm height) filled with water to a height of 12 cm (Fig. 2B). Following drug pretreatment, the animals (n = 12–13 in each group) were individually placed in the center of the tank, and video-recorded from the top view for 6 min, using Ethovision XT7 to calculate the distance traveled (m), average velocity (m/s) and meandering (°/m), as defined in [62]. Since zebrafish establish robust preferred loci (homebases) in the OFT [63], the homebase behavior was examined in this study in detail by comparing zebrafish activity in their preferred homebase quadrant with averaged activity in non-homebase quadrants (see details in Fig. 2B). Homebase quadrant was defined for each fish as described in [63,64], calculating the average time spent, frequency of visit and distance traveled in homebase vs. non-homebase quadrants. In addition, thigmotaxis behavior (preference for walls vs. center) was assessed in this study by virtually dividing the OFT arena into two zones – periphery (area within 2.5 cm from the walls) and the central arena. Using Ethovision XT7, the time spent, distance traveled, average velocity and frequency of visits were calculated for each zone in this test. Given previous rodent data and zebrafish data on glutamatergically