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Research report

Characterization of behavioral and endocrine effects of LSD on zebrafish

Leah Grossman[1], Eli Utterback[1], Adam Stewart, Siddharth Gaikwad, Kyung Min Chung, Christopher Suciu, Keith Wong, Marco Elegante, Salem Elkhayat, Julia Tan, Thomas Gilder, Nadine Wu, John DiLeo, Jonathan Cachat, Allan V. Kalueff*

Department of Pharmacology, Neuroscience Program and Zebrafish Neuroscience Research Consortium (ZNRC), Tulane University Medical School, 1430 Tulane Ave., New Orleans, LA 70112, USA

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ABSTRACT

Lysergic acid diethylamide (LSD) is a potent hallucinogenic drug that strongly affects animal and human behavior. Although adult zebrafish (*Danio rerio*) are emerging as a promising neurobehavioral model, the effects of LSD on zebrafish have not been investigated previously. Several behavioral paradigms (the novel tank, observation cylinder, light–dark box, open field, *T*-maze, social preference and shoaling tests), as well as modern video-tracking tools and whole-body cortisol assay were used to characterize the effects of acute LSD in zebrafish. While lower doses (5–100 μg/L) did not affect zebrafish behavior, 250 μg/L LSD increased top dwelling and reduced freezing in the novel tank and observation cylinder tests, also affecting spatiotemporal patterns of activity (as assessed by 3D reconstruction of zebrafish traces and ethograms). LSD evoked mild thigmotaxis in the open field test, increased light behavior in the light–dark test, reduced the number of arm entries and freezing in the *T*-maze and social preference test, without affecting social preference. In contrast, LSD affected zebrafish shoaling (increasing the inter-fish distance in a group), and elevated whole-body cortisol levels. Overall, our findings show sensitivity of zebrafish to LSD action, and support the use of zebrafish models to study hallucinogenic drugs of abuse.

1. Introduction

Lysergic acid diethylamide (LSD) is the most potent known hallucinogenic drug [1–4]. Despite a long history of LSD research, the mechanisms of its action are complex and poorly understood [1,4–6]. The drug acts on several neurotransmitter systems, modulating various serotonin [5,7–12] and dopamine [1,13–18] receptors. The clinical effects of LSD are also complex, and range from anxiety/panic and mood swings, to hyperactivity/euphoria, depersonalization, hallucinations [1,2,6,15,19,20], altered social behavior and memory [1,21–23].

LSD has been extensively tested in rodents, affecting their agonistic behaviors [24–27], sensorimotor gating [10], exploration and locomotion [5,28–30]. LSD exerts complex context-specific effects on animal social behaviors and cognition, including social aggression [26,27,31], memory and learning [30,32,33]. The drug has a characteristic biphasic action on rodent behavior, which includes initial anxiety and hypoactivity followed by hyperlocomotion [9,18,26,29,34–37]. Central serotonin 5-HT1a, 2a, 2c, and 5a [7,8,28,34,37,38] and dopamine D1, 2 and 4 [17,18] receptors, have been shown to contribute to LSD behavioral effects.

Soon after its discovery, LSD was tested in fish, evoking surface swimming, nose-up/tail-down position and hypolocomotion in beta splenders, guppies, neons, carps, minnows and goldfish [23,39–47]. However, these early studies focused on general assessment of fish locomotion, and did not evaluate other behavioral domains. A recent resurge of interest in LSD research [1,6,21,48–50] requires novel approaches, tools and animal models to better understand the effects of this drug on the brain and behavior.

The zebrafish (*Danio rerio*) is rapidly becoming a popular model species for neurobehavioral and pharmacology research [51–55]. These fish exhibit robust behavioral responses, their genome is well-characterized, and they contain homologous neural and endocrine systems to humans [56,57]. Previous research has utilized zebrafish as models sensitive to pharmacological manipulations affecting humans and rodents [52,53,55,58]. Since LSD effects have not yet been reported in this model, here we examine in-depth the behavioral and physiological effects of LSD on adult zebrafish.

2. Methods

2.1. Animals and housing

Total 530 adult (5–7 month-old) male and female "wild type" (short fin) zebrafish were obtained from a local commercial distributer (50 Fathoms,

* Corresponding author at: Department of Pharmacology, Room 3731, Tulane University Medical School, 1430 Tulane Ave., New Orleans, LA 70112, USA. Tel.: +1 504 988 3354; fax: +1 504 988 5283.
E-mail address: avkalueff@gmail.com (A.V. Kalueff).
[1] These two authors contributed equally to this study.

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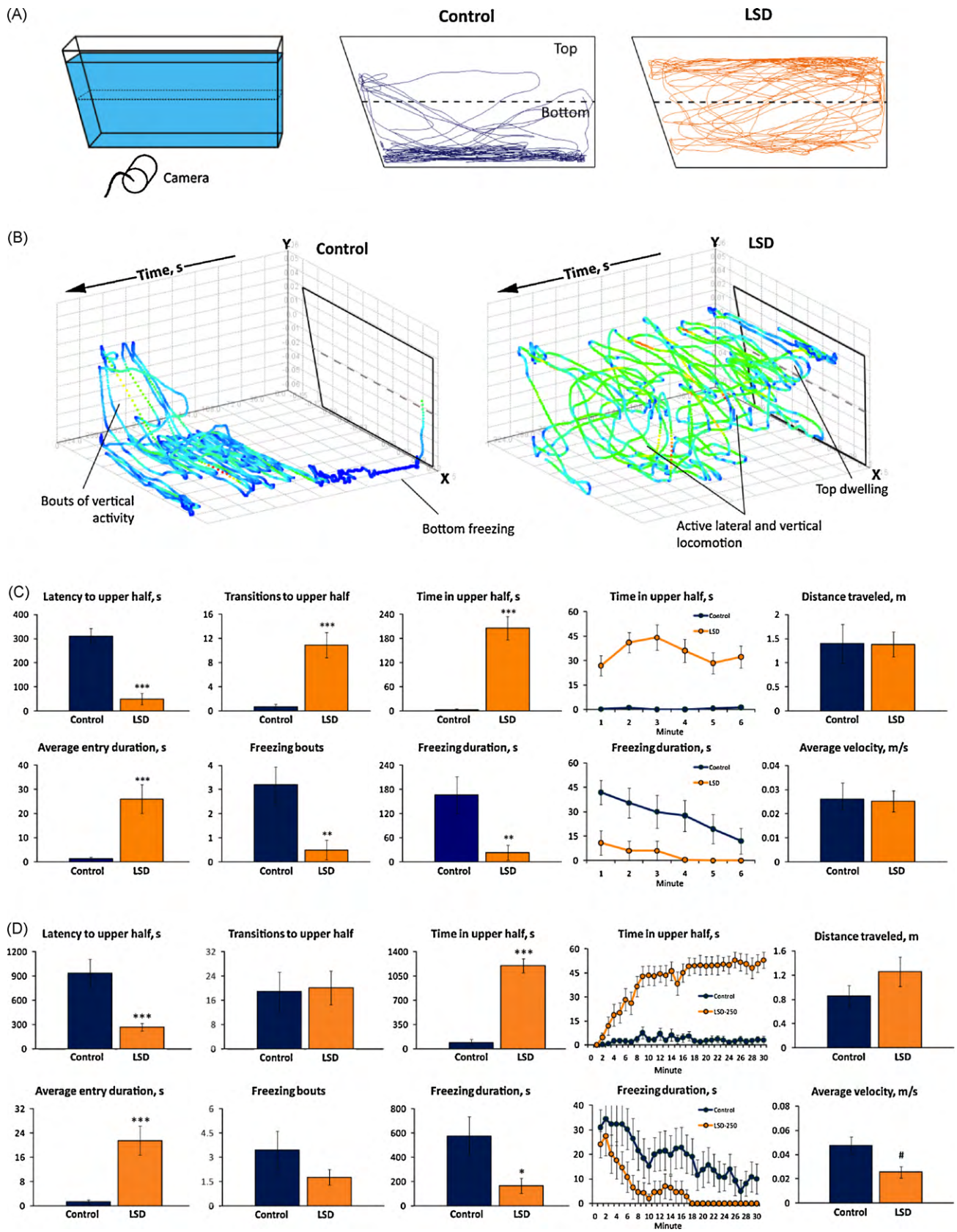

Fig. 1. Behavioral effects of LSD (250 μg/L) on zebrafish tested in the novel tank test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{\#}P = 0.05$–0.1 (trend) vs. control, *U*-test. (A–C) Standard 6-min novel tank test (Experiment 1, $n = 10$–12 per group) following a 20-min pre-treatment with LSD. Representative traces (A) at the bottom left of this panel were generated by Noldus Ethovision XT7 software using the side view video-recording. The three-dimensional reconstructions of zebrafish behaviors (B) were obtained by plotting zebrafish traces across the time of the test (see Section 2 for details). In all these experiments, the traces were examined for each experimental cohort, rated from 1 to n (based on similarity to each other), and the middle trace was selected as representative, to illustrate the patterns of zebrafish locomotion. (D) 30-min Novel tank test (Experiment 2, $n = 15$ in each group) with LSD solution added to the novel tank water.

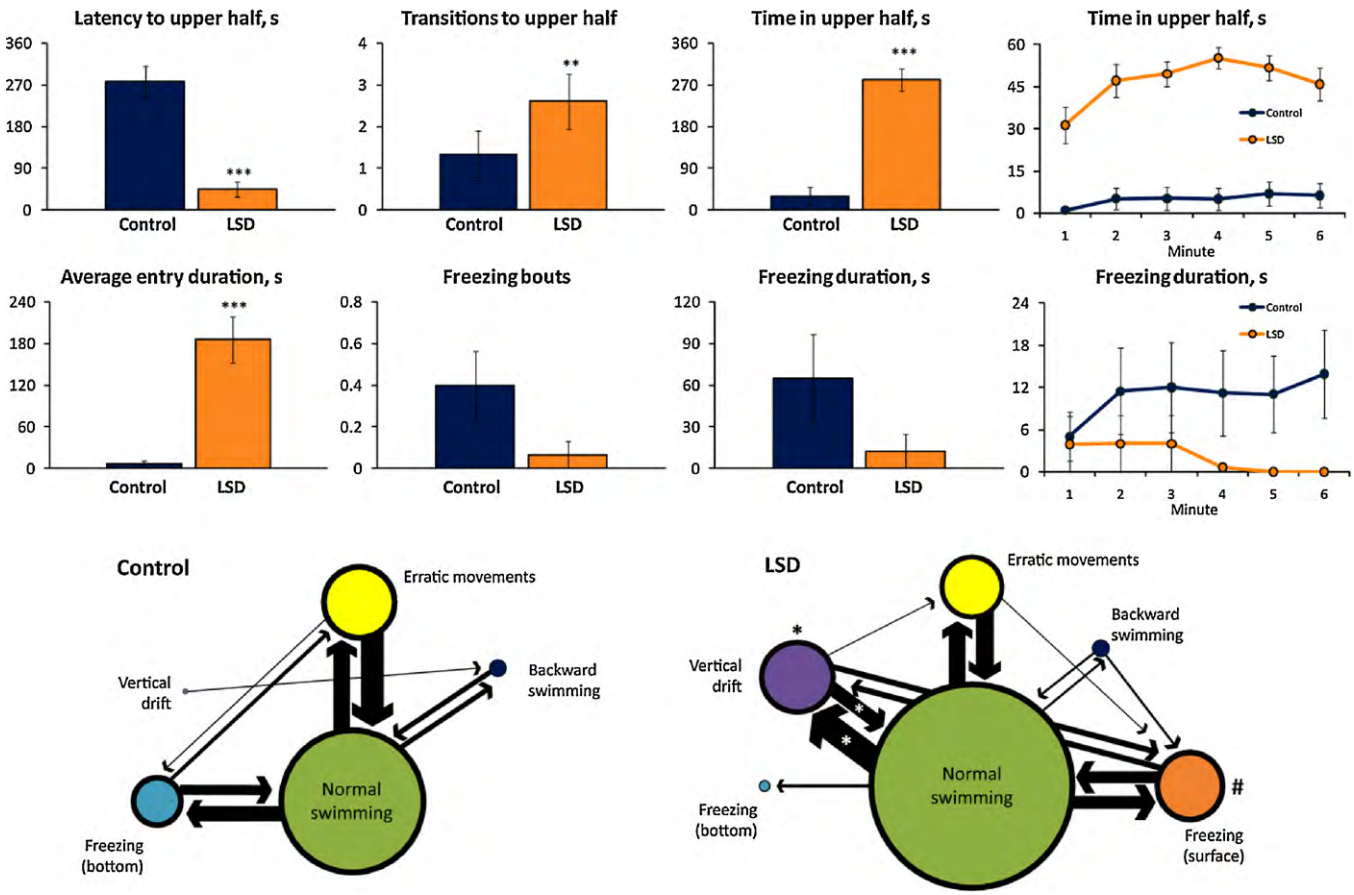

Fig. 2. Effects of LSD (250 μg/L) on zebrafish behavior (top) and its patterning (ethograms, bottom) in the 6-min observation cylinder ($n = 15$ in each group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{\#}P = 0.05–0.1$ (trend) vs. control, *U*-test. Ethograms were generated based on frequencies and transitions between each individual behavioral activity. The diameter of each circle corresponds to the frequency of each individual behavioral activity; the arrow width and direction reflect the frequency of transitions between these behaviors. Asterisks next to the circles denote significant differences vs. the respective control fish behaviors; asterisks placed on top of arrows indicate significant differences in the respective transitions (compared to the respective controls).

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. The tanks were filled with filtered (facility) water and maintained at 25–27 °C. Illumination (1000–1100 lx) was provided by ceiling-mounted fluorescent light tubes on a 12-h cycle (on: 6.00 h, off: 18.00 h) according to the standards of zebrafish care [59]. 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, USA), and immediately dissected on ice for further analysis.

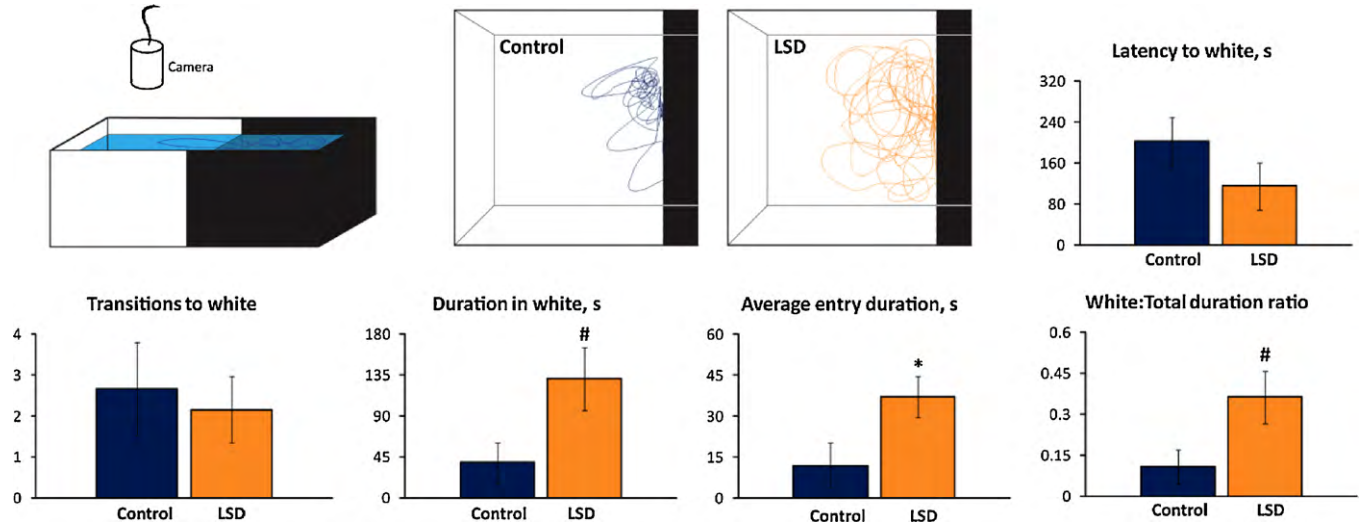

Fig. 3. Summary of behavioral effects of LSD (250 μg/L) on zebrafish tested in the 6-min light–dark box test ($n = 12$ in each group, Experiment 4). Representative traces were generated by Ethovision XT7 software using the top view video-recording; only light part of the box and a small part of the dark part are shown in this panel. $^{*}P < 0.05$, $^{\#}P = 0.05–0.1$ (trend) vs. control, *U*-test.

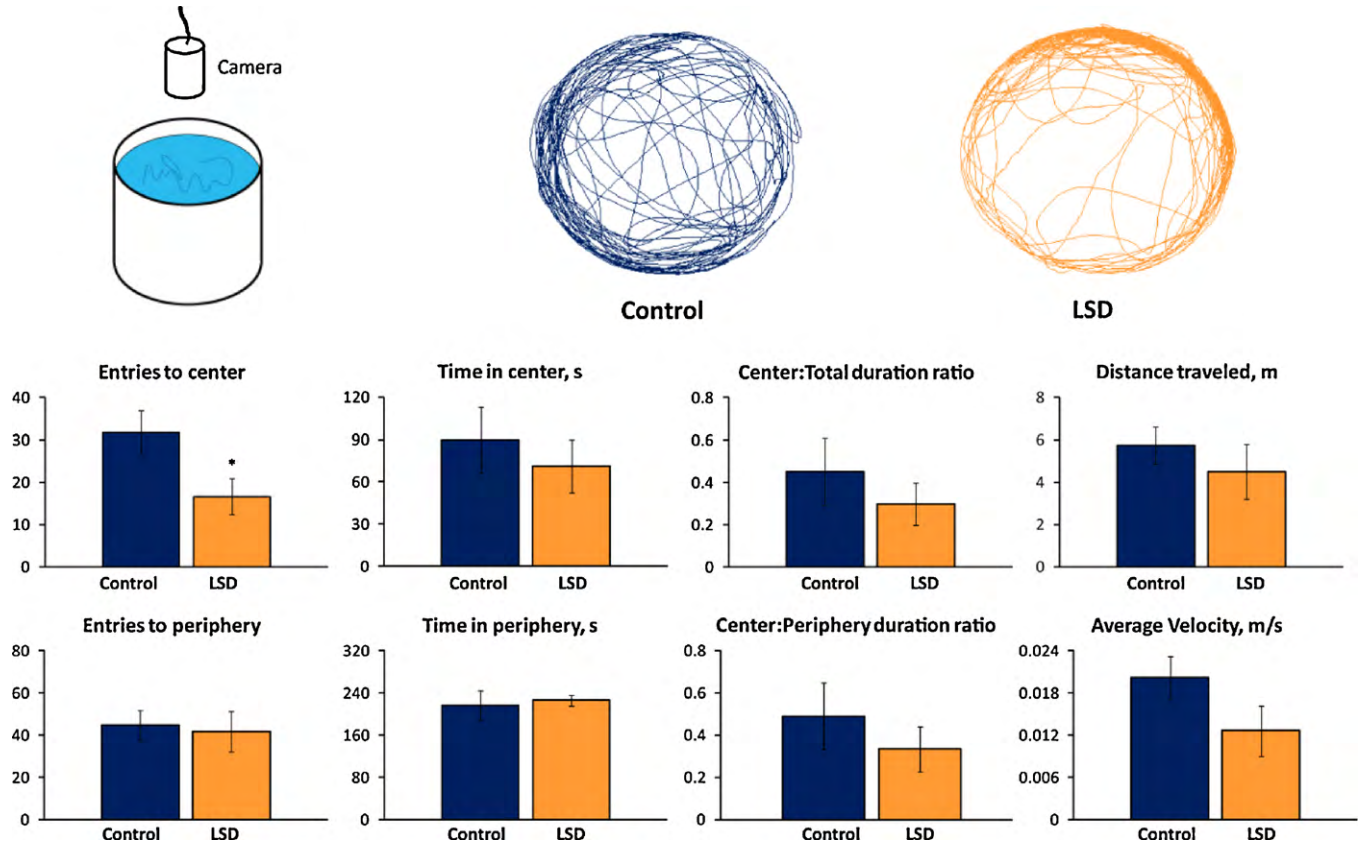

Fig. 4. Summary of behavioral effects of LSD (250 μg/L) on zebrafish tested in the 20-min open field test ($n = 15$ in each group, Experiment 5). Representative traces (bottom panel) were generated by Noldus Ethovision XT7 software using the top view video-recording. $^*P < 0.05$ vs. control, *U*-test.

2.2. Behavioral testing and apparatuses

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 six different behavioral tests, including the novel tank, observation cylinder, light–dark box, open field, *T*-maze, social preference and shoaling tests. To avoid the test battery effect, each test was performed on a separate cohort of naïve fish. All apparatuses rested on a level, stable surface. Zebrafish behavior was recorded by trained observers (inter-rater reliability >0.85), manually scoring different behavioral endpoints with subsequent analysis of traces by Ethovision XT7 (Noldus Information Technology, Netherlands).

The novel tank test, used to assess zebrafish anxiety and locomotion [53,60,61], was a 1.5-L trapezoidal tank (15 cm height × 28 cm top × 23 cm bottom × 7 cm width; Aquatic Habitats, Apopka, FL; Fig. 1A) maximally filled with water and divided into two equal virtual horizontal portions, by line marking the outside walls [53]. In Experiment 1, fish ($n = 10$–12 in each group) were pre-exposed to LSD for 20 min, and tested either in the standard, 6-min novel tank test. Experiment 2 targeted rapid LSD action, and exposed zebrafish ($n = 15$ in each group) to a 30-min novel tank filled with drug-treated water. 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 entries to the top, average entry duration (s), 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 or longer. Trials were also recorded to a computer using a USB webcam (2.0-Megapixel, Gigaware, UK) and subsequently analyzed by Ethovision XT7, assessing distance travelled (m) and velocity (m/s).

The observation cylinder test (Experiment 3) was used to assess zebrafish activity, similar to the novel tank test. The apparatus represented a 2-L glass cylinder (12 cm diameter, 19 cm height) maximally filled with water and divided horizontally into two equal virtual portions. Since LSD effects on behavioral organization were reported in rodents [25,28], this test also examined the microstructure (patterning) of zebrafish spontaneous behavior. Fish ($n = 15$ in each group) were pre-treated with LSD or a vehicle, and placed individually in the cylinder, and observed for 6 min, scoring the latency to top (s), time spent in top (s), number of top entries, average entry duration (s), as well as the number and duration (s) of freezing bouts. Videos were re-analyzed manually off-line, recording the sequence of the following behavioral episodes: normal swimming, vertical drift (passive trans-like vertical motion), backward swimming, bouts of erratic movement, bottom freezing, nose-up/tail-down surface freezing, and horizontal surface freezing. These endpoints were selected based on the normal zebrafish behavioral repertoire (e.g., normal swimming, bottom freezing, erratic movements [53]) as well as represented specific phenotypes evoked in other fish species by LSD [39–47] or in zebrafish by other hallucinogenic drugs [58]. This data was then used to create ethograms (Fig. 2)—visual diagrams that reflect frequencies and transitions between each individual behavioral activity (e.g., [62,63]) and characterize overall patterning of animal behavior. Ethograms for both LSD-treated and control fish were generated in this experiment. The diameter of each circle in the ethograms corresponds to the frequency of each individual behavioral activity, while the arrow width and direction reflect the frequency of transitions between these behaviors (Fig. 2).

The light–dark test (Experiment 4), based on the natural preference of zebrafish for dark environments [54,64], 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 (Fig. 3), demarcated by black and white coloration [54]. Fish ($n = 12$ in each group) were individually introduced into the black half (facing the wall), and video-recorded for 6 min, scoring 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). To further characterize zebrafish light–dark preference, the white:total time spent ratios were calculated for both cohorts.

The open field test (Experiment 5), conceptually similar to rodent open field test [54,65], consisted of a white plastic cylinder (21 cm diameter, 24 cm height, Fig. 4) filled with water to a height of 12 cm. The bottom of the tank was divided into two virtual zones—center and periphery (the area within 5 cm from the walls). Following a pre-treatment, the animals ($n = 15$ in each group) were individually placed in the center of the tank, and recorded for 6 min, using Ethovision XT7 to calculate the time spent (s), distance travelled (m), and the number of visits to predefined central and peripheral zones. To further characterize zebrafish thigmotaxis, the center:periphery time spent ratios were calculated for both groups.

Spontaneous exploration of zebrafish was also assessed in the *T*-maze apparatus (Experiment 6), a clear acrylic T-shaped box with a 10 × 10 cm central area and three 20-cm arms (10 cm width, 10 cm height; Ezra Scientific, San Antonio, TX, Fig. 5A), maximally filled with water. The maze was not used here to study memory in zebrafish, but rather served as an additional novel arena to assess animal activity and exploration. Following a pre-treatment, zebrafish ($n = 12$ in each group) were introduced individually to the bottom arm of the *T*-maze (facing the wall) and observed for 6 min by trained observers, scoring the number of center and total arm entries, as well as the number of freezing episodes and their duration (s).

The social preference test (Experiment 7) examined zebrafish social behavior and locomotor activity, similar to the mouse social preference paradigm [66,67].

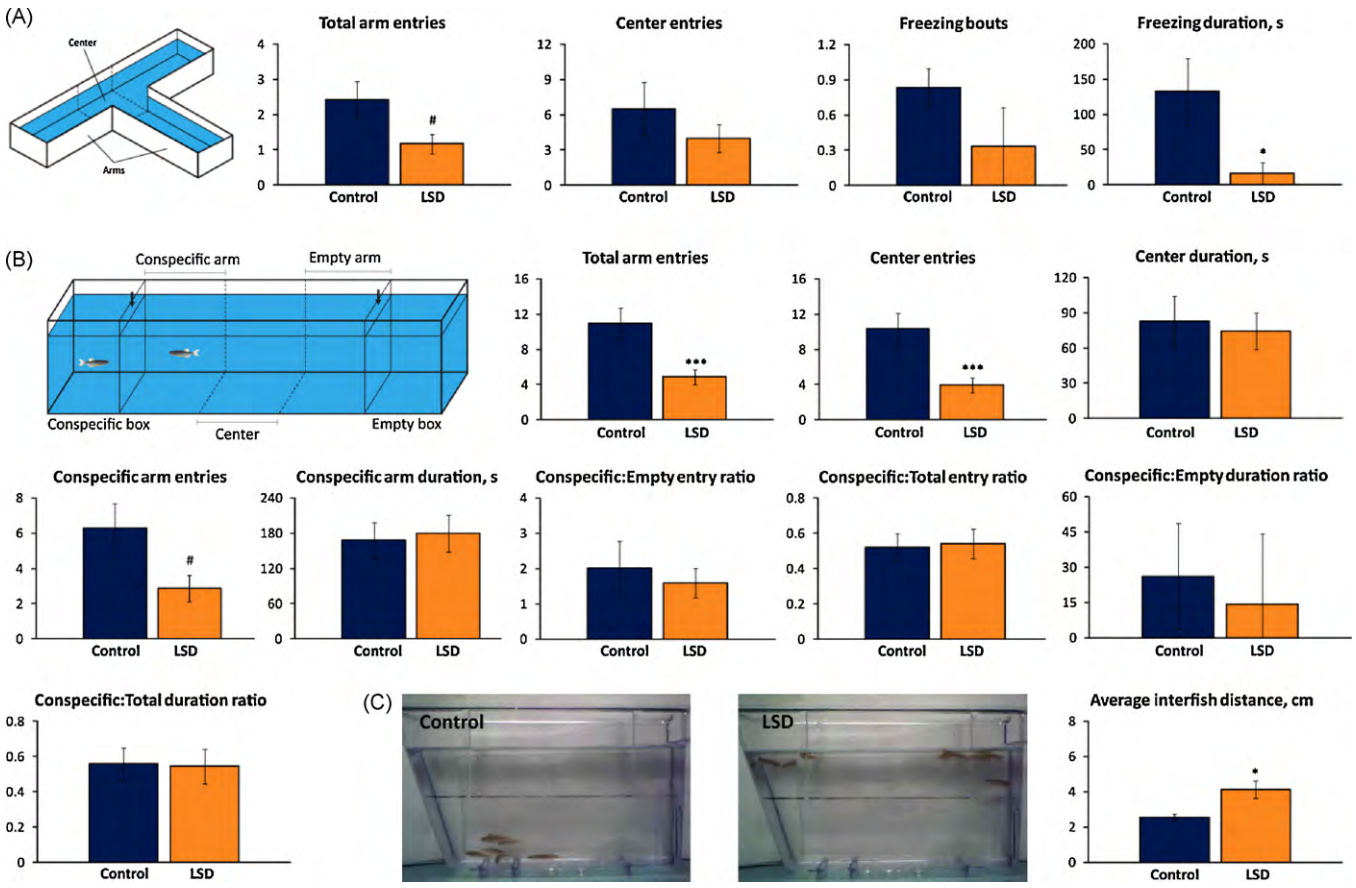

Fig. 5. Behavioral effects of LSD (250 μg/L) on zebrafish tested in the 6-min *T*-maze and social preference tests. $^{*}P < 0.05$, $^{\#}P = 0.05$–0.1 (trend) vs. control, *U*-test. (A) The *T*-maze test ($n = 12$ in each group, Experiment 6). (B) The social preference test ($n = 10$ in each group, Experiment 7). Small arrows indicate the transparent sliding doors, serving as dividers between the apparatus' arms and conspecific or empty box. (C) The shoaling test ($n = 16$ in each cohort, Experiment 8).

The apparatus was modified from the *T*-maze described above, by blocking the bottom arm with the sliding door, resulting in a 50-cm Plexiglas corridor (Fig. 5B). The target *conspecific* fish was introduced to an exposure compartment (conspecific box), separated by transparent sliding doors from the rest of the apparatus. To avoid lateral bias in zebrafish cohorts, the left/right location of target fish alternated between the trials. After a 20-min pre-treatment, control and LSD zebrafish ($n = 10$ in each group) were introduced individually to the central zone, temporarily separated by sliding doors from the two arms of the corridor. Following a 30-s interval (to reduce transfer/handling stress), the two sliding doors were gently lifted, and zebrafish was released to explore the apparatus for 6 min. Fish behavior was scored manually by trained observers, assessing the number of center entries, time spent in center (s), the number of "conspecific" arm entries, the number of "non-conspecific" (empty) arm entries, total arm entries, as well as time spent (s) in the respective zones of the apparatus. The ratios of conspecific:empty and conspecific:total entry and time spent was calculated based on this data.

The shoaling test (Experiment 8, Fig. 5C) was performed to examine the effects of LSD on social behavior of zebrafish shoals, given early reports on LSD modulation of shoaling in neons [3,68] and recent zebrafish data on shoaling sensitivity to various drugs [69,70]. Groups of 8 zebrafish were pre-exposed to either LSD (2 groups, $n = 16$) or water (2 groups, $n = 16$) for 20 min, and group-tested in the novel tank. Zebrafish shoaling behavior was video-recorded for 6 min, and analyzed using 8 screenshots made every 20 s during the last half of the observation period. A total of 16 screenshots from LSD-treated cohorts, and 16 screenshots from control cohorts were used for analyses in this study. Each screenshot was properly calibrated and analyzed by trained observers, manually measuring the distances (cm) between each fish in the group, and then averaging this data to obtain an average inter-fish distance per screenshot (final shoaling data for control and experimental cohorts represented averaged results for 16 screenshots per group).

2.3. Video-tracking and track reconstruction

Recorded videos were analyzed with Ethovision XT7 software, as described previously [52,55]. All arenas were calibrated across the bottom and walls of the tanks, and the calibration axes were placed to designate the origin (0,0) at the center of each tank. Behavioral data were then exported to Excel to generate total and per-minute plots for each endpoint and each group. The track data for each fish was exported as raw data into separate spreadsheets, providing spatial coordinates and endpoint values for each fish across a time scale broken down into fractions of a second. The exported traces were independently rated from 1 to *n* (based on similarity to each other) by 2 trained observers (inter-rater reliability >0.85), on a consensus basis. The middle trace was selected as representative for the group, to illustrate the pattern of exploration. Spatiotemporal 3D reconstructions were created for the novel tank test (Fig. 1B) with a Scatter 3D Color plotter, in which the *X* center, *Y* center and time were plotted on the *X*-, *Y*- and *Z*-axis, respectively, and the final images were exported using screen capture software (Debut Video Capture, NCH Software, Australia). Again, the generated 3D traces were rated from 1 to *n* (as described above), and the middle trace was selected as representative for the group.

2.4. Pharmacological manipulations

LSD doses and treatment time were chosen based on previous studies using LSD in fish [42,44–46] and our pilot studies to determine the effective dose range in zebrafish. While LSD at 5, 25, 50 and 75 μg/L did not affect fish behavior in the novel tank test, 100 μg/L produced only non-significant trends (data not shown). In contrast, 250 μg/L evoked marked behavioral responses (Fig. 1), justifying the utility of this dose for probing LSD effects in zebrafish tested here. In all experiments except Experiment 2, the drug pre-treatment was performed by submerging zebrafish into a 3-L plastic beaker containing 250 μg/L of LSD, for 20 min prior to the testing. In Experiment 2, LSD treatment was performed by dosing the 250 μg/L directly to the novel tank prior to testing. Control fish were exposed to drug-free water in all these experiments.

2.5. Whole-body cortisol assay

Whole-body samples were taken from fish used in Experiments 1, 4 and 5. Individual body samples obtained from experimental and control cohorts were homogenized in 500 μL of ice-cold 1× PBS buffer. The homogenizing rotor blade was then washed with an additional 500 μL of PBS and collected in a 2-mL tube containing the homogenate. Samples were transferred to glass extract-O tubes and cortisol was extracted twice with 5 mL of diethyl ether (Fisher Scientific, USA). After ether evaporation, the cortisol was reconstituted in 1 mL of 1× PBS. To quantify cortisol concentrations, ELISA was performed using a human salivary cortisol

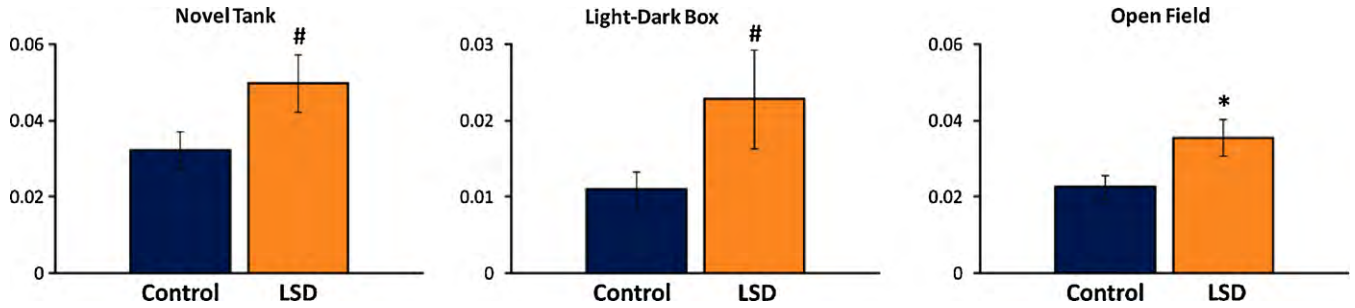

Fig. 6. Effects of LSD (250 μg/L) on zebrafish whole-body cortisol concentrations (ng/g body weight) measured using human salivary cortisol ELISA. $^{*}P < 0.05$, $^{\#}P = 0.05–0.1$ (trend) vs. control, *U*-test.

assay kit (Salimetrics LLC, State College, PA) [53]. ELISA plates were measured in a VICTOR-WALLAC plate reader using the manufacturer's software package. Whole-body cortisol levels were determined using a 4-parameter sigmoid minus curve fit based on the absorbencies of standardized concentrations, and presented as relative concentrations per gram of body weight for each fish [53].

2.6. Statistical analysis

The experimental data was analyzed using the Mann–Whitney *U*-test. Data were expressed as mean ± SEM. Significance was set at $P < 0.05$.

3. Results

In the 6-min novel tank test (Fig. 1), 250 μg/L LSD produced significantly shorter latency to enter the top, less freezing, and markedly more transitions, time spent in top and longer average entry duration. The distance travelled and velocity was unaffected in this study. Representative traces (Fig. 1A) clearly demonstrate active top swimming in LSD-treated fish, compared to the predominant bottom dwelling in controls. A detailed 3D reconstruction revealed altered spatiotemporal characteristics of swimming in LSD-treated fish (Fig. 1B). Control fish dove to the bottom, froze there and then gradually increased the amplitude of their activity, first in horizontal, and later in vertical, dimensions. This pattern, typical for normal zebrafish behavior [53,55,60], was markedly affected by LSD that caused fish to swim in both dimensions with a high amplitude and prevalence of top dwelling.

Experiment 2 assessed the immediate effects of the drug using a 30-min novel tank test filled with LSD-treated water. While most behaviors were similar to those observed in Experiment 1 in the 6-min tests, the LSD affected zebrafish in this test almost immediately, within several minutes (Fig. 1D). There were neither rapid anxiogenic nor pronounced behavioral inhibition-like effects, as LSD-treated zebrafish displayed lower freezing duration throughout this test (also see similar results in other tests used here).

In the observation cylinder test, LSD produced similar top dwelling and somewhat lowered freezing behavior in LSD-treated fish (Fig. 2). A detailed analysis of ethograms in this test revealed altered behavioral patterning in LSD-treated fish, which included additional behaviors and transitions typically not seen in normal control fish (Fig. 2).

In the light–dark box, the LSD-treated zebrafish spent more time (trend) and had significantly higher average entry durations to the light half (Fig. 3). Similar trends were observed for higher light:total time spent ratio. Representative traces, shown in Fig. 3, further illustrate higher light activity in LSD group in this test.

Fig. 4 shows behavioral effects of LSD in the open field test. While the drug did not affect distance travelled or velocity, it evoked thigmotaxis, significantly reducing center dwelling. Representative traces also confirm higher peripheral activity in LSD-treated fish (Fig. 4).

In the *T*-maze test, 250 μg/L LSD tended to reduce the number of arm entries or center entries and significantly lowered freezing duration and frequency (Fig. 5A). In the social preference test, LSD significantly reduced the number of total arm entries, center, con-conspecific and empty entries, but did not influence zebrafish social preference ratios (Fig. 5B). In the shoaling test, LSD disrupted normal shoaling behavior by significantly increasing the average inter-fish distance (Fig. 5C). Finally, LSD significantly elevated whole-body cortisol levels in fish tested in Experiment 5, with the similar trends for Experiments 1 and 4 (Fig. 6).

4. Discussion

Zebrafish-based paradigms are becoming increasingly popular in behavioral neuroscience and pharmacology research [71–73]. Although LSD has been studied in various animals, including fish [39–47], relatively little is known about LSD effects on fish behavior. Our study is the first report assessing LSD effects on zebrafish. We used a wide spectrum of behavioral paradigms to target multiple domains, from anxiety and activity to behavioral organization and social preference. In addition, behavioral testing was combined with modern video-tracking tools and a neuroendocrine (cortisol) assay, to reveal complex effects of LSD on zebrafish behavior and physiology.

Overall, LSD induced strong behavioral effects on zebrafish in all tests used here. The drug evoked robust top dwelling in zebrafish, reversed dark preference, induced thigmotaxis, altered spatiotemporal patterns of locomotion, reduced freezing duration, altered shoaling behavior, reduced the number of arm entries in *T*-maze and social preference test, but did not alter their social preference. LSD-treated zebrafish generally swam in a calmer and slower fashion, without frequent freezing bouts intermixed with erratic/darting behavior and rapid change of direction (typical for control zebrafish in novelty tests). The fact that LSD-exposed fish were moving constantly with minimal freezing resulted in unaltered distance travelled and average velocity. In the novel tank and observation cylinder, LSD rapidly increased top dwelling, generally paralleling data in other fish species [39,40,44,45]. In contrast to early fish studies [41,44,45,74], LSD-treated zebrafish were not immobile, as demonstrated by unaltered distance travelled, significantly reduced freezing (Figs. 1A and B and 3A), and 3D reconstruction of their locomotion (Fig. 1B). The latter finding is important in showing that LSD markedly alters overall 3D topography of zebrafish locomotion. Notably, a similar phenotype was seen in zebrafish chronically treated with fluoxetine [53], or exposed to its high acute doses (own unpublished observations). Therefore, this response may be serotonergically mediated, and induced by elevated serotonin or its agonists (such as LSD).

Although LSD has complex biphasic effects in rodents and humans [5,8,37,38], our experiments failed to detect anxiety

responses in zebrafish (Fig. 1C and D). The exact reasons why anxiety phase of the biphasic LSD profile was not observed here, require further studies. For example, this may be due to species differences for the roles that various neurotransmitter receptors, targeted by LSD, play in specific behaviors. Similarly, since hallucinogenic drugs alter sensory functions [10], it is possible that top dwelling observed in fish (Figs. 1–5) [42,43,46] reflects hallucinogenic effects of LSD. Given similar effects on zebrafish produced by opioid hallucinogens [58], this possibility seems indeed likely. Furthermore, while increased light behavior in the light–dark test (Fig. 3) can be due to higher locomotion (e.g., increasing chances of fish being in the light compartment), zebrafish did not make more entries to the light part. This phenotype is inconsistent with hyperactivity, but can be explained by distorted perception. The thigmotaxic response in zebrafish open field test (Fig. 4) parallels the effects of LSD and other hallucinogens in rodents [9], suggesting that it may be a common behavioral profile of hallucinogenic drugs in different species.

Interestingly, LSD produced conflicting effects on human [21,22] and animal [24–27] social behavior, with positive, negative and no effects reported in the literature. In our study, despite fewer conspecific arm entries in the social preference test, LSD globally reduced arm entries (Fig. 5A and B), which, together with unaltered conspecific:total or conspecific:empty arm ratios and time spent in the conspecific arm, suggests altered spatial exploration but unaltered social preference. Shoaling is also relevant to zebrafish social behavior. The fact that LSD has been reported to disorganize shoaling in other fish [3,68], and the sensitivity of zebrafish shoaling to various psychotropic drugs [69,70,75], emphasize the importance of this phenotype. In our study social preference was not affected, but shoaling was (Fig. 5C), suggesting that various social behaviors in zebrafish can be differentially modulated by LSD. Since disrupted shoaling behavior may reflect LSD hallucinogenic action, other tests, such as social hierarchy, dominance and boldness [76–78], may be used in future studies to dissect the LSD effects on zebrafish social and motor behavior.

Finally, LSD induced higher levels of cortisol in zebrafish. Analyzing this phenotype in zebrafish, it should be noted that LSD similarly activates the endocrine axis and elevates corticoids in humans [79] and animals [80,81]. Although cortisol positively correlates with zebrafish anxiety [52,53], overt anxiogenic responses were not observed in this study. Given this, and because the serotonergic system (especially via 5-HT_{1A} receptors) tightly controls the endocrine axis in teleost fish [82], it is possible that elevated cortisol in zebrafish may be due to central modulation of the fish endocrine system by LSD.

There were several additional limitations of this study. For example, complex dose-and time-dependent effects on behavior have been reported for LSD in various rodent studies [9,34]. Therefore, testing LSD doses both acutely and chronically, as well as using a longer observation time, may enable further characterization of LSD effects in zebrafish. Receptor mediation of the observed behaviors also requires an in-depth investigation, using selective agonists and antagonists. Given mounting rodent evidence [7,9,28,38], targeting a wider spectrum of behavioral repertoire, as well as sex and strain differences in zebrafish sensitivity to LSD, merit further scrutiny. Furthermore, other hallucinogens [4], such as mescaline, phencyclidine, lisuride or 3,4-methylenedioxymethamphetamine (MDMA), can be tested. For example, LSD and MDMA exert similar behavioral effects in zebrafish (own unpublished observations), confirming the utility of these fish for testing various hallucinogenic drugs.

In summary, LSD evokes marked physiological and behavioral responses in zebrafish that parallel some of its effects on humans and rodents. In line with recent studies on other hallucinogens [58], our data strongly support the utility of adult zebrafish for drug abuse research.

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References

[1] Pacifici R, Zuccaro P, Farre M, Pichini S, Di Carlo S, Roset PN, et al. The pharmacology of lysergic acid diethylamide: a review. CNS Neurosci Ther 2008;14(4):295–314.
[2] Cohen S. Psychotomimetic agents. Annu Rev Pharmacol 1967;7:301–18.
[3] Siegel RK. Hallucinogens and attentional dysfunction: a model for drug effects and reality testing. In: Stillman RC, Willette RE, editors. The psychopharmacology of hallucinogens. Elmsford, NY: Pergamon Press; 1978. p. 268–296.
[4] Nichols DE. Hallucinogens. Pharmacol Ther 2004;101(2):131–81.
[5] Backstrom JR, Chang MS, Chu H, Niswender CM, Sanders-Bush E. Agonist-directed signaling of serotonin 5-HT2C receptors: differences between serotonin and lysergic acid diethylamide (LSD). Neuropsychopharmacology 1999;21(2 Suppl):77S–81S.
[6] Gonzalez-Maeso J, Sealfon SC. Psychedelics and schizophrenia. Trends Neurosci 2009;32(4):225–32.
[7] Grailhe R, Waeber C, Dulawa SC, Hornung JP, Zhuang X, Brunner D, et al. Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. Neuron 1999;22(3):581–91.
[8] Gresch PJ, Strickland LV, Sanders-Bush E. Lysergic acid diethylamide-induced Fos expression in rat brain: role of serotonin-2A receptors. Neuroscience 2002;114(3):707–13.
[9] Palenicek T, Hlinak Z, Bubenikova-Valesova V, Novak T, Horacek J. Sex differences in the effects of N,N-diethyllysergamide (LSD) on behavioural activity and prepulse inhibition. Prog Neuropsychopharmacol Biol Psychiatry 2010;34(4):588–96.
[10] Halberstadt AL, Geyer MA. LSD but not lisuride disrupts prepulse inhibition in rats by activating the 5-HT(2A) receptor. Psychopharmacology (Berl) 208(2):179–89.
[11] Mittman SM, Geyer MA. Effects of 5HT-1A agonists on locomotor and investigatory behaviors in rats differ from those of hallucinogens. Psychopharmacology (Berl) 1989;98(3):321–9.
[12] Reissig CJ, Eckler JR, Rabin RA, Winter JC. The 5-HT1A receptor and the stimulus effects of LSD in the rat. Psychopharmacology (Berl) 2005;182(2):197–204.
[13] Gonzalez-Maeso J, Weisstaub NC, Zhou M, Chan P, Ivic L, Ang R, et al. Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. Neuron 2007;53(3):439–52.
[14] Marona-Lewicka D, Nichols DE. Complex stimulus properties of LSD: a drug discrimination study with alpha 2-adrenoceptor agonists and antagonists. Psychopharmacology (Berl) 1995;120(4):384–91.
[15] Jerome, D-Lysergic acid diethylamid investigator's brochure; 2008.
[16] Seeman P, Guan HC, Hirbec H. Dopamine D2High receptors stimulated by phencyclidines, lysergic acid diethylamide, salvinorin A, and modafinil. Synapse 2009;63(8):698–704.
[17] Marona-Lewicka D, Chemel BR, Nichols DE. Dopamine D4 receptor involvement in the discriminative stimulus effects in rats of LSD, but not the phenethylamine hallucinogen DOI. Psychopharmacology (Berl) 2009;203(2):265–77.
[18] Marona-Lewicka D, Thisted RA, Nichols DE. Distinct temporal phases in the behavioral pharmacology of LSD: dopamine D2 receptor-mediated effects in the rat and implications for psychosis. Psychopharmacology (Berl) 2005;180(3):427–35.
[19] Eveloff HH. The LSD syndrome. A review. Calif Med 1968;109(5):368–73.
[20] Levy RM. Diazepam for L.S.D. intoxication. Lancet 1971;1(7712):1297.
[21] Sigafoos J, Green VA, Edrisinha C, Lancioni GE. Flashback to the 1960s: LSD in the treatment of autism. Dev Neurorehabil 2007;10(1):75–81.
[22] Simmons 3rd JQ, Leiken SJ, Lovaas OI, Schaeffer B, Perloff B. Modification of autistic behavior with LSD-25. Am J Psychiatry 1966;122(11):1201–11.
[23] Siegel RK. Studies of hallucinogens in fish, birds, mice and men: the behavior of "psychedelic" populations. Adv Neuropsychopharmacol. Proceedings of the Symposium on VII Congress; 1971. p. 311–18.
[24] Krsiak M. Timid singly-housed mice: their value in prediction of psychotropic activity of drugs. Br J Pharmacol 1975;55(1):141–50.
[25] Geyer MA, Light RK. LSD-induced alterations of investigatory responding in rats. Psychopharmacology (Berl) 1979;65(1):41–7.
[26] Uyeno ET, Benson WM. Effects of lysergic acid diethylamide on attack behavior of male albino mice. Psychopharmacologia 1965;7(1):20–6.
[27] Silverman AP. Barbiturates, lysergic acid diethylamide, and the social behaviour of laboratory rats. Psychopharmacologia 1966;10(2):155–71.
[28] Krebs-Thomson K, Paulus MP, Geyer MA. Effects of hallucinogens on locomotor and investigatory activity and patterns: influence of 5-HT2A and 5-HT2C receptors. Neuropsychopharmacology 1998;18(5):339–51.
[29] Mittman SM, Geyer MA. Dissociation of multiple effects of acute LSD on exploratory behavior in rats by ritanserin and propranolol. Psychopharmacology (Berl) 1991;105(1):69–76.

[30] Castellano C. Effects of LSD-25 on avoidance behavior and locomotor activity in mice. Psychopharmacology (Berl) 1979;62(2):145–9.
[31] Krsiak M. Effects of drugs on behaviour of aggressive mice. Br J Pharmacol 1979;65(3):525–33.
[32] Frederick DL, Gillam MP, Lensing S, Paule MG. Acute effects of LSD on rhesus monkey operant test battery performance. Pharmacol Biochem Behav 1997;57(4):633–41.
[33] Chaplygina SR. The effect of lysergic acid diethylamide on memory in mice. Zh Vyssh Nerv Deiat Im I P Pavlova 1975;25(1):66–9.
[34] Adams LM, Geyer MA. A proposed animal model for hallucinogens based on LSD's effects on patterns of exploration in rats. Behav Neurosci 1985;99(5):881–900.
[35] Adams LM, Geyer MA. LSD-induced alterations of locomotor patterns and exploration in rats. Psychopharmacology (Berl) 1982;77(2):179–85.
[36] Gupta. An examination of the effect of central nervous system stimulant and anti-depressant drugs on open field performance in rats. Eur J Pharmacol 1971;13:341–6.
[37] Krebs-Thomson K, Geyer MA. The role of 5-HT(1A) receptors in the locomotor-suppressant effects of LSD: WAY-100635 studies of 8-OH-DPAT, and LSD in rats. Behav Pharmacol 1996;7(6):551–9.
[38] Geyer MA, Light RK, Rose GJ, Petersen LR, Horwitt DD, Adams LM, et al. A characteristic effect of hallucinogens on investigatory responding in rats. Psychopharmacology (Berl) 1979;65(1):35–40.
[39] Abramson HA, Evans LT. Lysergic Acid Diethylamide (Lsd-25). 34. Comparison with Effect of Psilocybin on the Siamese Fighting Fish. J Psychol 1963;56:363–74.
[40] Gettner HH, Rolo A, Abramson HA. Lysergic acid diethylamide (Lsd-25). Xxxv. Comparison of effect on siamese fighting fish and goldfish. J Psychol 1964;58:57–63.
[41] Abramson HA, Gettner HH, Carone PA, Rolo A, Krinsky L. The intracranial injection of drug in goldfish. I: Hallucinogens and their antagonism to smooth muscle activity. J Asthma Res 1979;16(2):55–61.
[42] Trout DL. Interaction of serotonin and lysergic acid diethylamide in the Siamese fighting fish. J Pharmacol Exp Ther 1957;121(1):130–5.
[43] Arbit J. Effects of LSD-25 upon Betta splendens: reliability of a bioassay technique. J Appl Physiol 1957;10(2):317–8.
[44] Abramson HA, Gettner HH, Hewitt MP, Dean G. Effect of lysergic acid diethylamide on the surfacing behaviour of large carp. Nature 1962;193:320–1.
[45] Chessick RD, Kronholm J, Maier G. Effect of lysergic acid diethylamide (Lsd-25)and other drugs on tropical fish. J Nerv Ment Dis 1963;137:389–94.
[46] Chessick RD, Kronholm J, Beck M, Maier G. Effect of pretreatment with tryptamine, tryptophan and dopa on Lsd reaction in tropical fish. Psychopharmacologia 1964;5:390–2.
[47] Abramson HA, Evans LT. Lysergic acid diethylamide (LSD 25). II. Psychobiological effects on the Siamese fighting fish. Science 1954;120(3128):990–1.
[48] Dyck E. Flashback: psychiatric experimentation with LSD in historical perspective. Can J Psychiatry 2005;50(7):381–8.
[49] Geyer MA. Why study hallucinogenic drugs in animals? Heffer Rev Psychedel Res 1998;1:33–8.
[50] Sessa B. Is it time to revisit the role of psychedelic drugs in enhancing human creativity? J Psychopharmacol 2008;22(8):821–7.
[51] Maximino C, de Brito TM, Dias CA, Gouveia A Jr., Morato S. Scototaxis as anxiety-like behavior in fish. Nat Protoc 5(2):209–16.
[52] Cachat J, Canavello P, Elegante M, Bartels B, Hart P, Bergner C, et al. Modeling withdrawal syndrome in zebrafish. Behav Brain Res 2010;208(2):371–6.
[53] Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF, et al. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav Brain Res 2009;205(1):38–44.
[54] Stewart A, Kadri F, DiLeo J, Chung K, Cachat J, Goodspeed J, et al. The developing utility of zebrafish in modeling neurobehavioral disorders. Int J Comp Psychol 2010;23:104–21.
[55] Wong K, Elegante M, Bartels B, Elkhayat S, Tien D, Roy S, Goodspeed J, et al. Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). Behav Brain Res 2010;208(2):450–7.
[56] Kaslin J, Panula P. Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (*Danio rerio*). J Comp Neurol 2001;440(4):342–77.
[57] Gabriel JP, Mahmood R, Kyriakatos A, Soll I, Hauptmann G, Calabrese RL, et al. Serotonergic modulation of locomotion in zebrafish: endogenous release and synaptic mechanisms. J Neurosci 2009;29(33):10387–95.
[58] Braida D, Limonta V, Pegorini S, Zani A, Guerini-Rocco C, Gori E, et al. Hallucinatory and rewarding effect of salvinorin A in zebrafish: kappa-opioid and CB1-cannabinoid receptor involvement. Psychopharmacology (Berl) 2007;190(4):441–8.
[59] Westerfield M. The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*). 5th ed. Eugene: University of Oregon Press; 2007.
[60] Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. Physiol Behav 2007;90(1):54–8.
[61] Bencan Z, Sledge D, Levin ED. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. Pharmacol Biochem Behav 2009;94(1):75–80.
[62] Denmark A, Tien D, Wong K, Chung A, Cachat J, Goodspeed J, et al. The effects of chronic social defeat stress on mouse self-grooming behavior and its patterning. Behav Brain Res 2010;208(2):553–9.
[63] Kalueff AV, Ren-Patterson RF, LaPorte JL, Murphy DL. Domain interplay concept in animal models of neuropsychiatric disorders: a new strategy for high-throughput neurophenotyping research. Behav Brain Res 2008;188(2):243–9.
[64] Serra EL, Medalha CC, Mattioli R. Natural preference of zebrafish (*Danio rerio*) for a dark environment. Braz J Med Biol Res 1999;32(12):1551–3.
[65] Choleris E, Thomas AW, Kavaliers M, Prato FS. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Biobehav Rev 2001;25(3):235–60.
[66] Crawley JN. Mouse behavioral assays relevant to the symptoms of autism. Brain Pathol 2007;17(4):448–59.
[67] Crawley JN. Designing mouse behavioral tasks relevant to autistic-like behaviors. Ment Retard Dev Disabil Res Rev 2004;10(4):248–58.
[68] Siegel RK. Intoxication: the universal drive for mind-altering substances. Lake Book Inc.; 2005. p. 372.
[69] Echevarria DJ, Hammack CM, Pratt DW, Hosemann JD. A novel behavioral test battery to assess global drug effects using the zebrafish. Int J Comp Psychol 2008;21(1):19–34.
[70] Gerlai R, Lee V, Blaser R. Effects of acute and chronic ethanol exposure on the behavior of adult zebrafish (*Danio rerio*). Pharmacol Biochem Behav 2006;85(4):752–61.
[71] Trompouki E, Zon LI. Small molecule screen in zebrafish and HSC expansion. Methods Mol Biol 2010;636:301–16.
[72] Arslanova D, Yang T, Xu X, Wong ST, Augelli-Szafran CE, Xia W. Phenotypic analysis of images of zebrafish treated with Alzheimer's gamma-secretase inhibitors. BMC Biotechnol 2010;10(1):24.
[73] Hortopan GA, Dinday MT, Baraban SC. Zebrafish as a model for studying genetic aspects of epilepsy. Dis Model Mech 2010;3(3–4):144–8.
[74] Weiss B, Abramson HA, Baron MO. Lysergic acid diethylamide (LSD-25). XXV. Effect of potassium cyanide and other oxidase and respiratory inhibitors on the Siamese fighting fish. AMA Arch Neurol Psychiatry 1958;80(3):345–50.
[75] Miller N, Gerlai R. Quantification of shoaling behaviour in zebrafish (*Danio rerio*). Behav Brain Res 2007;184(2):157–66.
[76] Larson ET, O'Malley DM, Melloni Jr RH. Aggression and vasotocin are associated with dominant-subordinate relationships in zebrafish. Behav Brain Res 2006;167(1):94–102.
[77] Paull GC, Filby AL, Giddins HG, Coe TS, Hamilton PB, Tyler CR. Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success. Zebrafish 2010;7(1):109–17.
[78] Wright D, Rimmer LB, Pritchard VL, Krause J, Butlin RK. Inter and intra-population variation in shoaling and boldness in the zebrafish (*Danio rerio*). Naturwissenschaften 2003;90(8):374–7.
[79] Bliss EL, Migeon CJ, Branch CH, Samuels LT. Reaction of the adrenal cortex to emotional stress. Psychosom Med 1956;18(1):56–76.
[80] Sackler AM, Weltman AS, Owens H. Effects of lysergic acid diethylamide on urinary 17-ketosteroid and 17-OH corticosteroid levels of female rats. Nature 1963;198:1119–20.
[81] Weltman AS, Sackler AM. Metabolic and endocrine effects of lysergic acid diethylamide (LSD-25) on male rats. J Endocrinol 1966;34(1):81–90.
[82] Winberg S, Nilsson A, Hylland P, Soderstom V, Nilsson GE. Serotonin as a regulator of hypothalamic–pituitary–interrenal activity in teleost fish. Neurosci Lett 1997;230(2):113–6.