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Behavioral and physiological effects of acute ketamine exposure in adult zebrafish

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ABSTRACT

Ketamine is a non-competitive glutamatergic antagonist used to induce sedation and analgesia. In sub-anesthetic doses, it induces hyperlocomotion, impairs memory and evokes stereotypic circling in rodents. Zebrafish (*Danio rerio*) emerged as a promising new animal model to screen the effects of psychotropic compounds. Here, we investigated the effects of sub-anesthetic doses of ketamine on anxiety, locomotion, habituation and social behavior of adult zebrafish. Acute 20-min exposure to 20 and 40 mg/L (but not 2 mg/L) of ketamine reduced anxiety, impaired intra-session habituation, evoked circular swimming and disrupted zebrafish shoaling. Additionally, ketamine reduced whole-body cortisol levels and elevated brain *c-fos* expression in zebrafish. Our findings demonstrate the sensitivity of zebrafish to behavioral and physiological effects of sub-anesthetic doses of ketamine, further supporting the utility of this species as a model for neuropharmacological research, including testing ketamine and related drugs.

1. Introduction

Ketamine is a sedative drug used in clinical and veterinary medicine to induce analgesia and anesthesia [14,31,84]. It acts as a non-competitive antagonist of N-methyl-D-aspartate (NMDA) glutamate receptors, and at high doses also modulates opioid, monoamine, cholinergic and γ-aminobutyric acid (GABA) receptors [23,26,38,42,44,60,81].

Due to its common use as an anesthetic drug, behavioral and physiological effects of ketamine have been studied extensively in various rodent models. In rodents, sub-anesthetic ketamine increases locomotion [39,86] and stereotypic behaviors, impairs social interaction [20,32], and exerts antidepressant or anxiolytic effects [29]. In humans, ketamine produces hallucinogenic, sedative [25,51], dissociative, pro-psychotic [50,59] and antidepressant effects [94]. However, the complex pharmacological profile of ketamine remains poorly understood, and warrants testing in new experimental paradigms and with novel model organisms.

Various aquatic species have been extensively used in psychopharmacology and neurotoxicology research [1,33,36,53,69]. The zebrafish (*Danio rerio*) is emerging as a promising high-throughput model for neurobehavorial research due to its well-characterized genome, robust behavioral responses, and physiological similarity to humans [55]. While *larval* screens have provided important insights into neuropharmacology of various agents [21,76], *adult* zebrafish models are becoming widely used due to their developed physiological systems and more complex behavior [17,73].

A recent study tested ketamine in adult zebrafish, showing aberrant circling behavior [93]. Together with the presence of functional NMDA receptors in zebrafish brain [22], this supports the zebrafish sensitivity to various NMDA antagonists (e.g., [77]). Here, we used several behavioral paradigms to further investigate the effects of acute ketamine exposure in zebrafish. To parallel behavioral and physiological responses, we also examined the effects of this drug on zebrafish neuroendocrine responses using a whole-body cortisol assay recently developed in our laboratory [18,28]. Finally, given the ability of ketamine and other hallucinogens to induce early proto-oncogene *c-fos* gene expression in rodents [43,63,67], and the similar actions of LSD and MDMA in zebrafish ([74] and own unpublished observations), we examined the effects of ketamine exposure on brain *c-fos* expression.

2. Methods

2.1. Animals and housing

A total of 183 adult (5–7 months) male and female wild type *short-fin* zebrafish were obtained from a local commercial distributor; (50 Fathoms, Metairie, LA) and housed in groups of 20–30 fish per 40-L tank, filled with filtered system water maintained at 25–27 °C. Illumination was provided by ceiling-mounted fluorescent light tubes

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on a 14:10-h cycle according to the standards of zebrafish care [83]. All fish used in this study were experimentally naïve, and fed twice daily [35]. Following behavioral testing, the animals were euthanized in 500 mg/L Tricaine (Sigma-Aldrich, St. Louis, MO). Animal experiments were approved by IACUC and adhered to National and Institutional guidelines and regulations.

2.2. Pharmacological manipulations

Ketamine for this study was obtained through Sigma-Aldrich (St. Louis, MO). Its doses (2, 20, and 40 mg/L) were chosen based on our pilot studies with the drug and extrapolated from previously published research on ketamine effects in zebrafish [93]. A standard 20-min pre-treatment time was chosen here based on our experience with ketamine and other similar hallucinogenic drugs [35,74,75], and was consistent with the time course of ketamine's effects reported in earlier rodent studies [39,80]. Based on our experience, this exposure time was also sufficient for physiological (e.g., cortisol and *c-fos*) responses of zebrafish to develop. Drug exposure in this study was performed by submerging the individual zebrafish in a 1-L plastic beaker for 20 min prior to the testing. The solution was regularly changed after each exposure to ensure that each fish is exposed to a consistent concentration of ketamine. Control fish were exposed to drug-free water for the same treatment time, as described above.

2.3. Behavioral testing

Behavioral testing was performed between 11.00 and 15.00 h using tanks filled with water adjusted to the holding room temperature. Zebrafish behavior was recorded by the same three highly trained observers (inter-rater reliability >0.85), who were blinded to the treatments and manually scored different behavioral endpoints with subsequent analysis of traces by Ethovision XT7 software (Noldus IT, Wageningen, Netherlands) [35].

The novel tank test (Experiment 1) was used to assess zebrafish behavior for 6 min following a 20-min pre-treatment with varying doses of ketamine (2, 20 and 40 mg/L). The novel tank test was a 1.5-L trapezoidal tank (15 height × 28 top × 23 bottom × 7 cm width; Aquatic Habitats, Apopka, FL) maximally filled with water and divided into two equal virtual horizontal portions (top and bottom), by a line marking the outside walls [28]. Zebrafish behavior was video-recorded by a side-view camera to calculate the latency (s) to the upper half (top), distance traveled (m), velocity (m/s), transitions (whole-body crossing into upper half) to, and time spent (s) in the top. Trained observers also scored manually the frequency of erratic movements (sharp changes in direction, high velocity, unorganized darting typically along bottom of tank), as well as the frequency and duration (s) of freezing bouts (total absence of movement, except for eyes and gills, for 2 s or longer), as described previously [28,35]. Anxiety-like states are typically accompanied with reduced top locomotion and increased bottom dwelling, erratic movements and freezing [28,35].

Once behavioral data was generated for each minute of the test in Experiment 1, we examined intra-session habituation (as an index of spatial working memory) of zebrafish behaviors by comparing behavioral scores for the first and the last minute (min 1 vs. min 6) of the novel tank trials, as described previously [88].

Given previous rodent data on ketamine and rotational behavior [61,62], we also analyzed zebrafish circling behavior, initially focusing on three possible types of rotations: i) circling behavior in the horizontal plane, ii) circling in the vertical axis, and iii) spiraling-like circling in the horizontal plane while moving vertically. As ketamine induced only horizontal circling in this model, this type of rotation behavior was further evaluated in the present study. For this, video track data generated by Noldus Ethovision XT7 were replayed in slow motion, to analyze zebrafish circling behavior and record the direction (right/left hand) of each complete turn. Since ketamine evoked characteristic tight circling in zebrafish, rotation was defined as a full 360° circle of ~5 cm (~2 fish lengths) in diameter. The data was then analyzed for total rotations, right-handed rotations, left-handed rotations, and left-handed:right-handed ratio. In addition, we assessed the number of fish demonstrating 'high rotation' behavior (defined as 5 or more full rotations per trial) in this study.

Since Experiment 1 identified the lowest dose of 2 mg/L as inactive (Fig. 1), only two higher doses (20 and 40 mg/L) of ketamine were used in Experiments 2–5. The light–dark test (Experiment 2) was a rectangular tank (15 height × 30 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 [73]. The light–dark test is based on zebrafish innate aversion of light areas, and is sensitive to anxiolytic manipulations which generally increase the number of entries to, and time spent in, the light [73]. Fish (n = 12 in each group) were exposed to ketamine (20 or 40 mg/L) or water (control). Zebrafish were individually introduced into the black half (facing the wall), and video-recorded by top-view camera for 6 min, scoring the latency to enter (s), time spent (s) and the number of entries into the white, as well as the average entry duration (s; time spent divided by the number of entries) [35]. Due to the dark background, zebrafish behavior in the black compartment was not assessed here.

The open field test (Experiment 3) represented a white plastic cylinder (21 diameter × 24 cm height) filled with water to a height of 12 cm. Following a 20-min pre-treatment with ketamine (20 and 40 mg/L) or water, the animals (n = 10–12 in each group) were individually placed in the center of the tank, and recorded by a top-view camera for 6 min, using Ethovision XT7 to calculate distance traveled (m) and velocity (m/s). In addition, rotation (circling) behaviors were examined in this study by trained observers analyzing traces of zebrafish swimming, similar to the novel tank test described earlier.

The social preference test (Experiment 4) consisted of a 50-cm Plexiglas corridor divided into five cells (10 × 10 cm). The target *conspecific* fish was introduced to an exposure compartment (conspecific box), separated by a transparent divider from the rest of the apparatus [35]. 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 ketamine-exposed zebrafish (n = 12 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 the zebrafish was released to explore the apparatus for 6 min. Fish behavior was scored manually by trained observers, assessing the number of entries and time spent (s) in center, conspecific arms or empty arms [35]. The ratios of conspecific:empty entries and duration were also calculated based on this data.

The shoaling test (Experiment 5) was performed to examine the effects of ketamine on social behavior of zebrafish shoals. Zebrafish are sociable animals and form shoals as they swim in various tanks [71,72,90,91]. Shoal cohesion is usually higher in more anxious animals, and can be measured by the average inter-fish distance for each shoal. Groups of 8 zebrafish (2 groups/16 fish per dose) were pre-exposed to ketamine (20 or 40 mg/L) or water (control) for 20 min, and group-tested in the novel tank, as described elsewhere [35]. Zebrafish shoaling behavior was video-recorded by a side-view camera for 6 min, and analyzed using 8 screenshots made every 20 s during the last 3 min of the observation period. A total of 16 screenshots from each group were assessed in this study. Each screenshot was calibrated and analyzed by trained observers, measuring the distances (m) between each fish in the group, and then averaging this data to obtain an average inter-fish distance per screenshot using ImageTool software (UTHSCSA, San Antonio, TX).

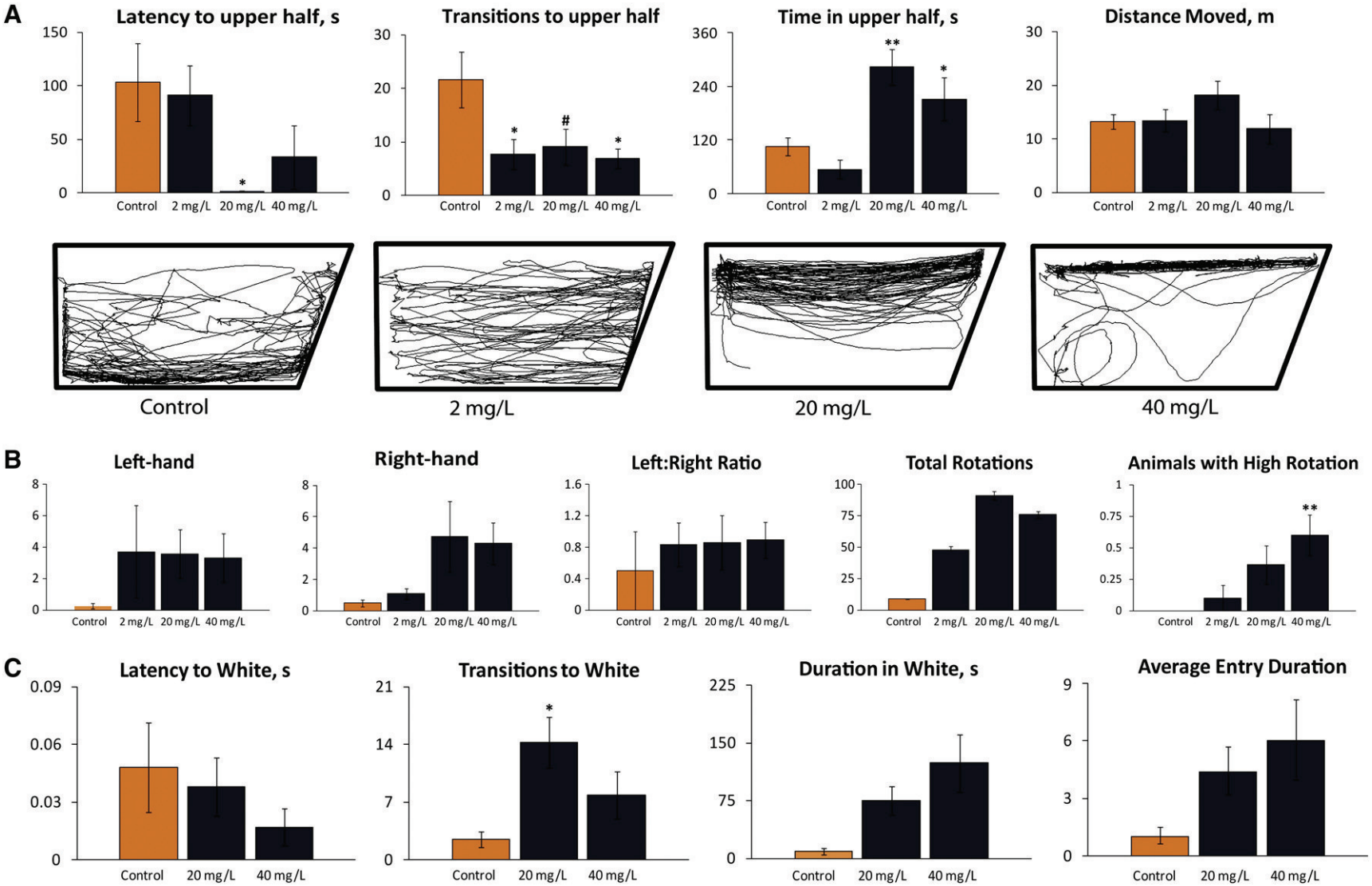

Fig. 1. Behavioral effects of 20-min acute exposure to ketamine on zebrafish tested in the novel tank and the light–dark box tests. A: Behavioral endpoints obtained in the 6-min novel tank test (n = 12 per group; Experiment 1). In the bottom row of this panel, representative 2D traces were generated by Noldus Ethovision XT7 software using the side view video-recording. In all 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. B: Rotation data obtained in the novel tank test (endpoints represent the average number of left, right or total rotations, the respective left:right ratio, and the average number of animals per group displaying high rotation behavior (5 or more full rotations) during the 6-min test). C: Behavioral endpoints obtained in the 6-min light–dark box test (n = 12 per group; Experiment 2). *P < 0.05, **P < 0.01, #P = 0.05–0.1 (trend) vs. controls; post-hoc Tukey test for significant ANOVA data.

The top/bottom preference was also assessed by counting the number of fish in top and bottom areas of the tank per screenshot. Final shoaling data for control and experimental cohorts represented averaged results for 8 screenshots per group.

2.4. Video-tracking

During manual observation, videos were recorded in MPEG1 format with the maximum sample rate 30 fps for each trial by auto-focusing 2.0 MP USB webcams, placed 50 cm in front of or on top of the tanks, and attached to laptop computers. Recorded videos were analyzed with Ethovision XT7 software, as described previously [16,35,88]. All environments were calibrated for each arena, and the calibration axes were placed to designate the origin (0,0) at the center of each tank. The track data for each fish was exported as raw data into separate spreadsheets. The exported traces were independently rated on a consensus basis from 1 to n (based on similarity to each other) by trained observers blinded to the treatments. The middle trace was selected as representative of the group to illustrate the spatial pattern of zebrafish swimming activity [35].

2.5. Whole-body cortisol and brain c-fos assays

Whole-body and whole brain samples for this study were obtained from fish tested previously in the novel tank and shoaling tests. Individual body samples obtained from experimental and control cohorts were homogenized in 1 mL of ice-cold 1× PBS buffer and transferred to glass extract-O tubes for cortisol extraction with 5 mL of diethyl ether. After ether evaporation, the cortisol was reconstituted in 1 mL of 1× PBS and quantified using a human salivary cortisol ELISA assay kit (Salimetrics LLC, State College, PA). Whole body cortisol levels were determined using a 4-parameter sigmoid minus curve fit, and presented as relative concentrations per gram of body weight for each fish [18].

QT-PCR was next performed for zebrafish *c-fos* mRNA. Whole brains were used in this study based on recent studies utilizing whole brains to examine genomic responses in adult zebrafish [48,52,82], and our own studies of drug-evoked *c-fos* expression in zebrafish [74,89]. The brains were dissected and 2–4 brains were pooled to obtain 6 samples per group [74]. RNA was extracted from these samples, and quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, DE). The cDNA was synthesized using random primers and iScript Select cDNA Synthesis Kit (Bio Rad, CA). For QT-PCR, cDNA was amplified with *c-fos* primers for zebrafish, and amplified products were compared against the reference gene, *elongation factor 1 alpha*, as described elsewhere [78]. *C-fos* data was expressed as the relative fold change vs. the respective control (taken as 100%).

2.6. Statistical analysis

The experimental data was analyzed using one-way ANOVA (factor: dose) followed by post-hoc Tukey testing for significant ANOVA data. Intra-session habituation in Experiment 1 was analyzed using the paired *U*-test to compare min 1 vs. min 6 data. *C-fos* expression for each dose was individually compared with the respective control group, and analyzed using non-paired *U*-test. Inter-rater reliability for the observers was determined by Spearman correlation. Data were expressed as mean ± SEM; significance was set at $P<0.05$.

3. Results

In the novel tank test, ANOVA revealed a significant drug effect on time spent in top ($F_{(3, 35)}=10.7$, $P<0.0001$), number of entries to top ($F_{(3, 35)}=3.8$, $P<0.05$), latency to top ($F_{(3, 35)}=3.1$, $P<0.05$), but not distance traveled, velocity, freezing bouts, freezing duration or the number of erratic movements (NS). Post-hoc analysis revealed significantly longer time spent in top induced by 20 and 40 mg/L of ketamine, shorter latency to top evoked by 20 mg/L, and fewer transitions to top in 2 and 40 mg/L, with a similar trend in 20 mg/L cohorts (Fig. 1A). Representative 2D traces clearly demonstrate active top swimming in ketamine-treated fish, compared to control zebrafish (Fig. 1A). Right-hand, left-hand and total rotations were generally higher in ketamine groups, although these effects did not reach significance by ANOVA (Fig. 1B). While no lateralization in the direction of rotations was found by calculating the left-hand:right-hand ratio (NS), there was a significant drug effect for the number of animals showing 'high rotation' phenotype ($F_{(3, 35)}=5.2$, $P<0.005$), with 40 mg/L of ketamine significantly increasing circling in zebrafish (Fig. 1B). Likewise, inter-trial habituation in the novel tank test was statistically significant for the number of entries to top and time spent in top in control zebrafish ($P<0.05$, min 1 vs. min 6, *U*-test), demonstrated a trend ($P=0.05$–0.1) for 2 mg/L, and was impaired by both 20 and 40 mg/L of ketamine (NS, min 1 vs. min 6).

In the light–dark box test, ketamine produced a significant effect on the number of transitions to white ($F_{(2, 35)}=5.6$, $P<0.001$) but not the latency to white, time spent in white or average entry duration (NS). Post-hoc analysis showed that the dose of 20 mg/L of ketamine evoked significantly more entries to the white, compared to control zebrafish (Fig. 1C).

In the open field test, there was no significant drug effect for distance traveled or velocity. Interestingly, like in the novel tank, analysis of zebrafish swim traces showed rotation movements (Fig. 2). Although right-hand, left-hand and total rotations were generally higher in ketamine-treated groups, these effects did not reach significance by ANOVA. There was neither overt lateralization in the direction of rotations, nor significant difference in the number of animals showing 'high rotation' phenotype (NS, Fig. 2).

In the social preference test, one-way ANOVA found significant drug effect for the number of entries to center ($F_{(2, 35)}=5.5$, $P<0.01$), conspecific arm ($F_{(2, 35)}=15.7$, $P<0.005$) and empty arm ($F_{(2, 35)}=18.1$, $P<0.005$), as well as time spent in the empty arm ($F_{(2, 35)}=9.5$, $P<0.05$). Both doses tested significantly increased entries into all three portions of the tank, with more time spent in the empty arm produced by 40 mg/L of ketamine (Fig. 3A). However, the ratios of conspecific:empty entries and time spent were not significantly affected in this test.

In the shoaling test, one-way ANOVA yielded significant drug effect on the inter-fish distance ($F_{(2, 47)}=64.8$, $P<0.05$) and top preference ($F_{(2, 47)}=23.7$, $P<0.05$). Both doses of ketamine tested significantly increased the average inter-fish distance and evoked top dwelling in zebrafish shoals (Fig. 3B).

Ketamine produced a significant effect on cortisol levels in the shoaling test ($F_{(2, 44)}=15.2$, $P<0.0001$), with a similar (albeit non-significant) profile in the novel tank test (Fig. 4A). Finally, the drug dose-dependently elevated *c-fos* expression in zebrafish tested in the novel tank, showing a trend towards a similar elevation in the shoaling test (Fig. 4B).

4. Discussion

Aquatic models are becoming increasingly popular in behavioral pharmacology and neurotoxicology research [7,40,41,79]. Despite the common use of ketamine in clinical and experimental studies, there are relatively few reports of its effects in fish [3,85], including zebrafish [93]. The current study tested sub-anesthetic doses of ketamine in several experimental models, demonstrating anxiolytic-like effects, impaired habituation, increased circling, reduced shoaling, lower cortisol levels and elevated *c-fos* brain expression, compared to water-treated controls (Figs. 1–4). While the low dose (2 mg/L) of ketamine was inactive in our study, the two higher doses

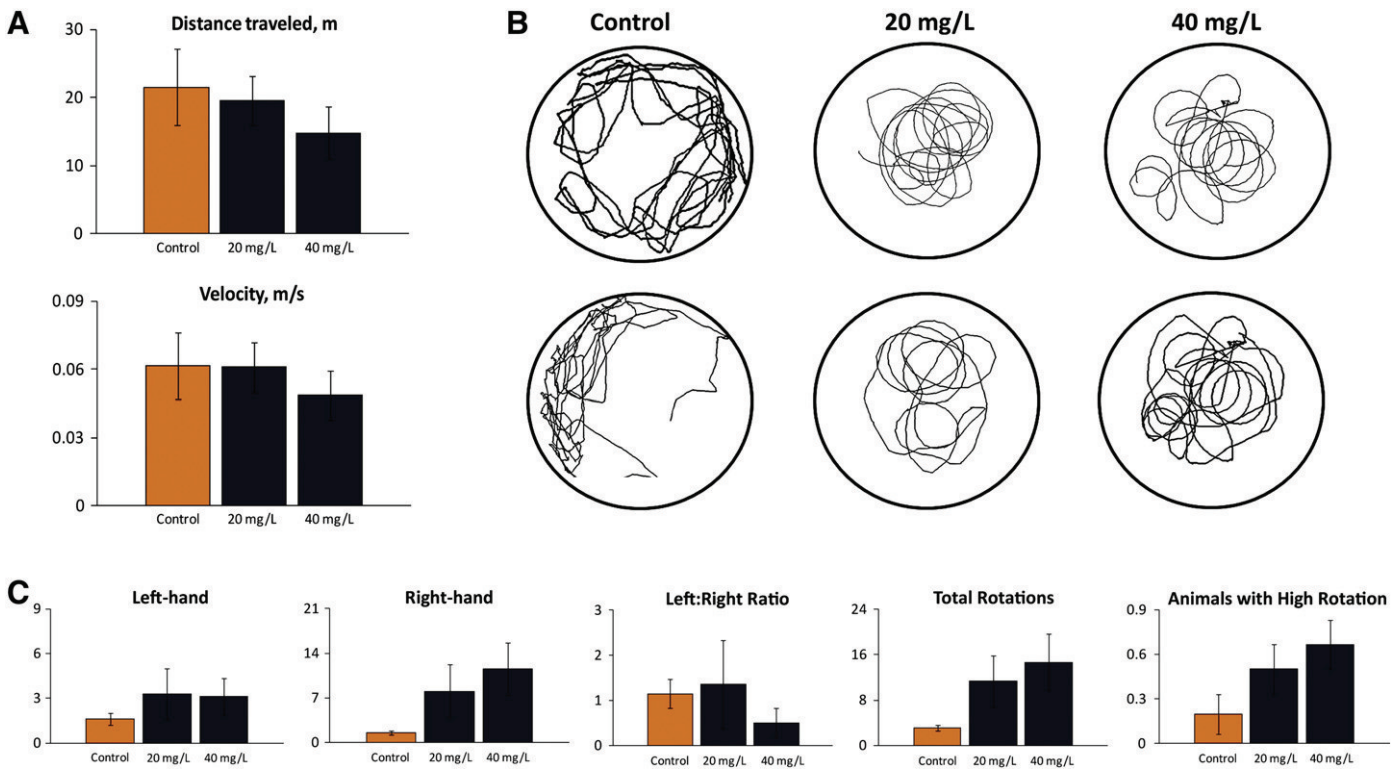

Fig. 2. Behavioral effects of 20-min acute exposure to ketamine on zebrafish tested in the open field test (Experiment 3; n = 10–12 per group). Behavioral data (A) and representative traces (B) were generated by Noldus Ethovision XT7 software using the side view video-recording. In all experiments, the 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. C: Rotation data obtained in the open field test (endpoints represent the average number of left, right or total rotations, the respective left:right ratio, and the average number of animals per group displaying high rotation behaviors (5 or more full rotations) during the 6-min test).

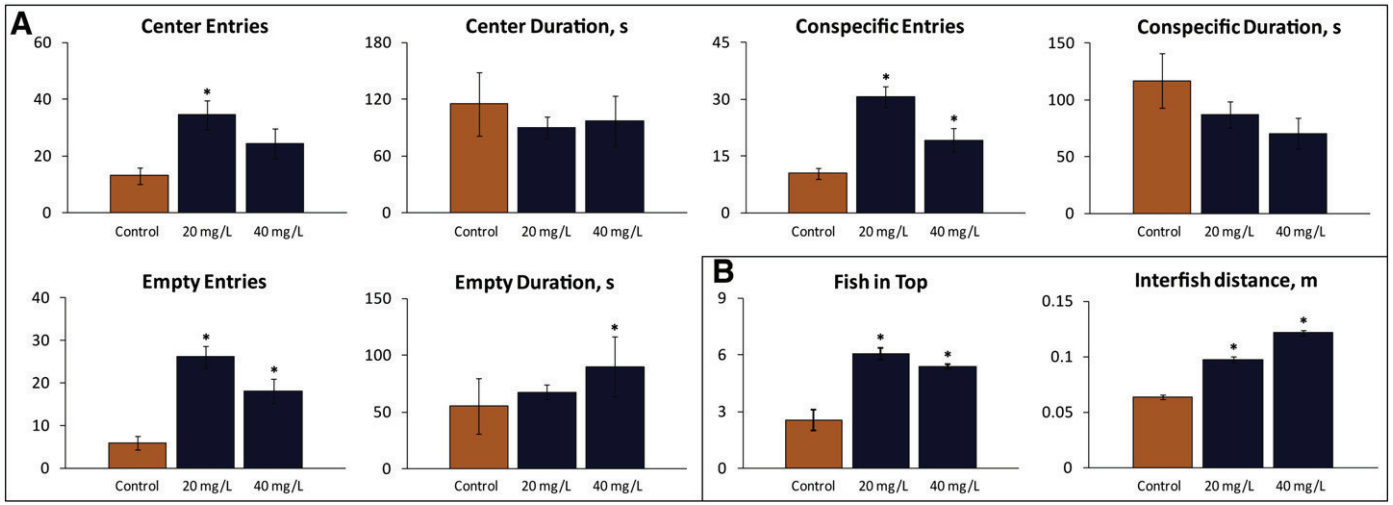

Fig. 3. Behavioral effects of 20-min acute exposure to ketamine on zebrafish tested in the social preference and shoaling tests. A: Standard 6-min social preference test (n = 12 per group; Experiment 4). Traditional endpoints in this test represent the average number and duration of entries to center, empty or conspecific arms. B: Standard 6-min shoaling test (n = 16 per group; Experiment 5). Traditional endpoints in this test represent the average number of fish in top and the average inter-fish distance. *P < 0.05 vs. controls; post-hoc Tukey test for significant ANOVA data.

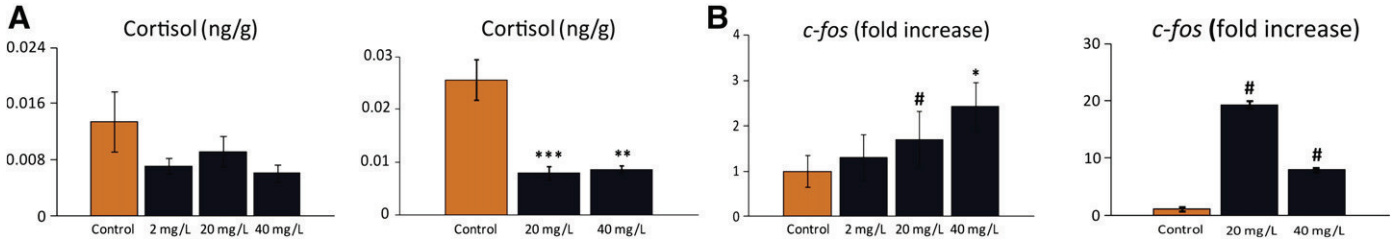

Fig. 4. Whole-body cortisol and brain *c-fos* expression levels in zebrafish exposed to ketamine for 20 min. A: Whole-body cortisol (ng/g body weight for each individual fish). B: Brain *c-fos* (fold increase relative to the respective controls, 6 pooled samples per group). Samples were collected from cohorts used in Experiment 1 (n = 12 per group, left diagram) and Experiment 5 (n = 16 per group, right diagram). For cortisol data in panel A: *P < 0.05, **P < 0.01 vs. controls, post-hoc Tukey test for significant ANOVA data. For *c-fos* data in panel B: *P < 0.05, #P < 0.05–0.1 (trend), *U*-test vs. respective controls.

(20, 40 mg/L) evoked anxiolytic-like responses in the novel tank and light–dark box tests (Fig. 1), resembling anti-anxiety effects produced by ketamine in rodents [19,87] and by MK-801 in zebrafish [27].

In addition, circling behavior was observed for this drug (Figs. 1 and 2), consistent with rotations previously reported for ketamine in zebrafish [93], and evoked by this drug [11,39,62,86] and other NMDA antagonists [4,30,37,80] in rodents. As already mentioned, circling in our study occurred only in the horizontal plane near the surface, without spiraling or vertical rotations, and was mostly observed in ketamine-treated fish (Figs. 1 and 2). Since a recent study noted lateralized circling in zebrafish, with a right-circling preference bias evoked by ketamine [93], we analyzed zebrafish swimming trajectories and the direction of their movement in the novel tank and open field tests. Our analysis (Figs. 1 and 2) showed that ketamine did not affect the direction of rotation (similar to the lack of lateral bias in circling evoked in zebrafish exposed to another NMDA antagonist, MK-801 [77]). Again, although rotations were generally higher in ketamine-treated groups in the novel tank and open field tests, these effects (except for the number of fish with 'high rotation' phenotype in the novel tank) did not reach significance by ANOVA, most likely due to high variance of these endpoints (Figs. 1 and 2). The fact that our study revealed milder circling (compared to [93]) may also be due to the difference in ketamine treatments (5-min with 2 g/L [93] vs. 20 min with lower doses of 20 and 40 mg/L in the present study). However, the similarity in the ketamine-evoked rotation between the two studies indicates that circling in zebrafish is indeed modulated by ketamine.

In general, one possibility for the circling phenotype in zebrafish may be due to hallucinogenic-like effects of ketamine (well-known to distort perception in humans [65]). However, other hallucinogenic drugs recently tested in zebrafish, such as lysergic acid diethylamide (LSD), 3,4-Methylenedioxymethamphetamine (MDMA), cocaine and salvinorin A ([35,74]; own unpublished data), did not evoke rotation in zebrafish. Together with circling behavior evoked in zebrafish by MK-801 [77], this raises the possibility that ketamine-induced rotation may be specific to NMDA antagonism, rather than hallucinogenic action in general. It is also possible that the observed responses in zebrafish are relevant to psychosis-like states induced by acute ketamine, known to trigger clinical and experimental psychoses [9,64] as well as hyperactivity and repetitive behaviors in humans [59] and rodents [9,19,61,62]. Further research will be needed to elucidate this problem, for example, by testing anti-psychotic drugs and their ability to modulate the effects of ketamine on zebrafish behavior.

Intra-session habituation was used in this study as a simple form of spatial working memory [88]. While control fish exhibited typical habituation of entries to top and time spent in top of the novel tank, ketamine at 20 m and 40 mg/L significantly impaired the habituation of these endpoints. Since ketamine can impair memory in rodents [2,5,58,60] and humans [50], our zebrafish data is consistent with this profile. However, given the link between memory and anxiety [46,47], the amnestic-like profile of ketamine may also be due to anxiolysis (known to reduce memory). Finally, similar memory-inhibiting effects were produced by other NMDA antagonists in rodents [60] and zebrafish [77], suggesting that glutamatergic modulation may contribute to the effects of ketamine on habituation observed here.

Additionally, zebrafish behavior was markedly altered in the shoaling and social preference tests. In the shoaling test, ketamine-treated zebrafish showed predominantly top swimming with dose-dependent increase in inter-fish distance (Fig. 3; see similar effect of MK-801 in the shoaling test [27]). While this may be interpreted as impaired social interaction (similar to its effects in rodents [8,19,49,70]), our social preference data show overall elevation in arm exploration in ketamine-treated fish, without an increase in social preference (Fig. 3A). Together with the fact that anxiolytic agents generally reduce shoal cohesion and increase top dwelling, this suggests that ketamine may not impair social behavior in zebrafish per se, but rather evokes behavioral activation and anxiolysis.

The reported anxiolytic profile of ketamine in several different behavioral paradigms (Figs. 1–3) was further supported by its effects on cortisol levels (Fig. 4). Ketamine tended to reduce whole-body cortisol in zebrafish, consistent with similar effects of other anxiolytics in zebrafish [17]. Finally, our results showed elevated brain *c-fos* expression produced by ketamine (Fig. 4). This response is consistent with behavior-activating properties of this drug, and strikingly similar to well-known ketamine-induced *c-fos* elevation in rodents [43,66].

Furthermore, there were several limitations of this study. For example, ketamine has a complex pharmacological profile that includes glutamatergic, monoaminergic, and GABA-ergic activity [6,51,54,68]. All these mediators are known to modulate activity and anxiety in a number of species, including zebrafish [10,12,15,34,46,54,77,92]. Therefore, the behavioral effects of ketamine in this study may not be limited to a single mechanism, such as NMDA antagonism. However, a similar anxiolytic and behavior-activating profile in zebrafish was reported for another NMDA antagonist, MK-801 [77]. Our own pilot experiments with MK-801 yielded anxiolytic responses in zebrafish novel tank, accompanied by reduced cortisol and elevated *c-fos* expression (own unpublished data). Similar to ketamine, circling was also evoked in both rodent and zebrafish by MK-801 [24,77], supporting the similarity between the behavioral and physiological effects of the two glutamatergic drugs on zebrafish. However, other mediators may also be involved, requiring further studies. For example, screening various NMDA agonists and antagonists, including chronic ketamine and its long-term effects, as well as co-administration of ketamine with other drugs (similar to rodent studies [62,86]) will be necessary. Likewise, testing a wider spectrum of behavioral domains, such as sensory functions, cognition and aggression, as well as age, sex and strain differences in zebrafish sensitivity to ketamine, merits further studies.

Finally, in addition to ketamine [15,93], mounting evidence indicates that zebrafish are sensitive to various other hallucinogenic drugs, such as cocaine [45,56,75], LSD [35,57], MDMA [74] and salvinorin A [13]. Complementing these reports, our present study further supports the utility of zebrafish for the screening of behavioral and physiological effects of ketamine and related anxiotropic hallucinogenic agents. Further studies are also needed to elucidate the mechanisms of ketamine action in zebrafish and other model species.

Conflict of interest

Nothing declared.

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References

[1] 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.
[2] Aguado L, San Antonio A, Perez L, del Valle R, Gomez J. Effects of the NMDA receptor antagonist ketamine on flavor memory: conditioned aversion, latent inhibition, and habituation of neophobia. Behav Neural Biol 1994];61:271–81.
[3] Al-Hamdani AH, Ebrahim SK, Mohammad FK. Experimental xylazine–ketamine anesthesia in the common carp *Cyprinus carpio*. J Wildl Dis 2010];46:596–8.
[4] Ali SF, Kordsmeier KJ, Gough B. Drug-induced circling preference in rats. Correlation with monoamine levels. Mol Neurobiol 1995];11:145–54.

[5] Amann LC, Halene TB, Ehrlichman RS, Luminais SN, Ma N, Abel T, et al. Chronic ketamine impairs fear conditioning and produces long-lasting reductions in auditory evoked potentials. Neurobiol Dis 2009];35:311–7.
[6] Ando Y, Hojo M, Kanaide M, Takada M, Sudo Y, Shiraishi S, et al. S(+)-Ketamine suppresses desensitization of gamma-aminobutyric acid type B receptor-mediated signaling by inhibition of the interaction of gamma-aminobutyric acid type B receptors with g protein-coupled receptor kinase 4 or 5. Anesthesiology 2011];114:401–11.
[7] Arslanova D, Yang T, Xu X, Wong ST, Augelli-Szafran, Xia W. Phenotypic analysis of images of zebrafish treated with Alzheimer's gamma-secretase inhibitors. BMC Biotechnol 2010];10(24).
[8] Becker A, Grecksch G. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Test of predictive validity. Prog Neuropsychopharmacol Biol Psychiatry 2004];28:1267–77.
[9] Becker A, Peters B, Schroeder H, Mann T, Huether G, Grecksch G. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2003];27:687–700.
[10] Bencan Z, Sledge D, Levin ED. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. Pharmacol Biochem Behav 2009];94:75–80.
[11] Bennett DA, Bernard PS, Amrick CL. A comparison of PCP-like compounds for NMDA antagonism in two in vivo models. Life Sci 1988];42:447–54.
[12] Benturquia N, Courtin C, Noble F, Marie-Claire C. Involvement of D1 dopamine receptor in MDMA-induced locomotor activity and striatal gene expression in mice. Brain Res 2008];1211:1–5.
[13] 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];190:441–8.
[14] Brown TC, Cole WH, Murray GH. Ketamine: a new anaesthetic agent. Aust N Z J Surg 1970];39:305–10.
[15] Burgess HA, Granato M. Sensorimotor gating in larval zebrafish. J Neurosci 2007];27:4984–94.
[16] Cachat J, Canavello P, Elegante M, Bartels B, Hart P, Bergner C, et al. Modeling withdrawal syndrome in zebrafish. Behav Brain Res 2010];208:371–6.
[17] Cachat J, Stewart A, Grossman L, Gaikwad S, Kadri F, Chung KM, et al. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat Protoc 2010];5:1786–99.
[18] Cachat J, Stewart A, Grossman L, Gaikwad S, Kadri F, Min Chung K, et al. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat Protoc 2010];5:1786–99.
[19] Canever L, Oliveira L, D'Altoe de Luca R, Correa PT, D. de BF, Matos MP, et al. A rodent model of schizophrenia reveals increase in creatine kinase activity with associated behavior changes. Oxid Med Cell Longev 2010];3:421–7.
[20] Canever L, Oliveira L, Luca RD, Correa PT, Fraga DD, Matos MP, et al. A rodent model of schizophrenia reveals increase in creatine kinase activity with associated behavior changes. Oxid Med Cell Longev 2010];3.
[21] Chen J, Patel R, Friedman TC, Jones KS. The behavioral and pharmacological actions of NMDA receptor antagonism are conserved in zebrafish larvae. Int J Comp Psychol 2010];23:82–90.
[22] Cox JA, Kucenas S, Voigt MM. Molecular characterization and embryonic expression of the family of N-methyl-D-aspartate receptor subunit genes in the zebrafish. Dev Dyn 2005];234:756–66.
[23] Crisp T, Perrotti JM, Smith DL, Stafinsky JL, Smith DJ. The local monoaminergic dependency of spinal ketamine. Eur J Pharmacol 1991];194:167–72.
[24] de Mello CF, Begnini J, Jimenez-Bernal RE, Rubin MA, de Bastiani J, da Costa Jr E, et al. Intrastriatal methylmalonic acid administration induces rotational behavior and convulsions through glutamatergic mechanisms. Brain Res 1996];721:120–5.
[25] Dickerson D, Pittman B, Ralevski E, Perrino A, Limoncelli D, Edgecombe J, et al. Ethanol-like effects of thiopental and ketamine in healthy humans. J Psychopharmacol 2010];24:203–11.
[26] Durieux ME. Inhibition by ketamine of muscarinic acetylcholine receptor function. Anesth Analg 1995];81:57–62.
[27] Echevarria DJ, Hammack CM, Pratt DW, Hosemann JD. A novel behavioral test battery to assess global drug effects using the zebrafish. Int J Compar Psychol 2008];21:19–34.
[28] 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];205:38–44.
[29] Engin E, Treit D, Dickson CT. Anxiolytic- and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models. Neuroscience 2009];161:359–69.
[30] Eshel Y, Korczyn AM. Circling behavior induced by phencyclidine in mice and its inhibition by naloxone. Experientia 1985];41:73–4.
[31] Finck AD, Ngai SH. Opiate receptor mediation of ketamine analgesia. Anesthesiology 1982];56:291–7.
[32] Gao XM, Elmer GI, Adams-Huet B, Tamminga CA. Social memory in mice: disruption with an NMDA antagonist and attenuation with antipsychotic drugs. Pharmacol Biochem Behav 2009];92:236–42.
[33] 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.
[34] Gonzalez-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF, et al. Identification of a serotonin/glutamate receptor complex implicated in psychosis. Nature 2008];452:93–7.
[35] Grossman L, Utterback U, Stewart A, Gaikwad S, Wong K, Elegante M, et al. Characterization of behavioral and endocrine effects of LSD on zebrafish. Behav Brain Res 2010];214:277–84.
[36] Grove DJ. The effects of adrenergic drugs on melanophores of the minnow, *Phoxinus phoxinus* L. Comp Biochem Physiol 1969];28:37–54.
[37] Haggerty GC, Forney RB, Johnson JM. The effect of a single administration of phencyclidine on behavior in the rat over a 21-day period. Toxicol Appl Pharmacol 1984];75:444–53.
[38] Hall R, Murdoch J. Brain protection: physiological and pharmacological considerations. Part II: The pharmacology of brain protection. Can J Anaesth 1990];37:762–77.
[39] Hetzler BE, Wautlet BS. Ketamine-induced locomotion in rats in an open-field. Pharmacol Biochem Behav 1985];22:653–5.
[40] Hill AJ, Teraoka H, Heideman W, Peterson RE. Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol Sci 2005];86:6–19.
[41] Hortopan GA, Dinday MT, Baraban SC. Zebrafish as a model for studying genetic aspects of epilepsy. Dis Model Mech 2010];3:144–8.
[42] Hustveit O, Maurset A, Oye I. Interaction of the chiral forms of ketamine with opioid, phencyclidine, sigma and muscarinic receptors. Pharmacol Toxicol 1995];77:355–9.
[43] Inta D, Trusel M, Riva MA, Sprengel R, Gass P. Differential c-Fos induction by different NMDA receptor antagonists with antidepressant efficacy: potential clinical implications. Int J Neuropsychopharmacol 2009];12:1133–6.
[44] Irifune M, Sato T, Kamata Y, Nishikawa T, Dohi T, Kawahara M. Evidence for GABA(A) receptor agonistic properties of ketamine: convulsive and anesthetic behavioral models in mice. Anesth Analg 2000];91:230–6.
[45] Irons TD, MacPhail RC, Hunter DL, Padilla S. Acute neuroactive drug exposures alter locomotor activity in larval zebrafish. Neurotoxicol Teratol 2010];32:84–90.
[46] Kalueff A, Nutt DJ. Role of GABA in memory and anxiety. Depress Anxiety 1996];4:100–10.
[47] Kalueff AV, Jensen CL, Murphy DL. Locomotory patterns, spatiotemporal organization of exploration and spatial memory in serotonin transporter knockout mice. Brain Res 2007];1169:87–97.
[48] Kily LJ, Cowe YC, Hussain O, Patel S, McElwaine S, Cotter FE, et al. Gene expression changes in a zebrafish model of drug dependency suggest conservation of neuro-adaptation pathways. J Exp Biol 2008];211:1623–34.
[49] Koros E, Rosenbrock H, Birk G, Weiss C, Sams-Dodd F. The selective mGlu5 receptor antagonist MTEP, similar to NMDA receptor antagonists, induces social isolation in rats. Neuropsychopharmacology 2007];32:562–76.
[50] Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994];51:199–214.
[51] Krystal JH, Perry Jr EB, Gueorguieva R, Belger A, Madonick SH, Abi-Dargham A, et al. Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function. Arch Gen Psychiatry 2005];62:985–94.
[52] Lefebvre KA, Tilton SC, Bammler TK, Beyer RP, Srinouanprachan S, Stapleton PL, et al. Gene expression profiles in zebrafish brain after acute exposure to domoic acid at symptomatic and asymptomatic doses. Toxicol Sci 2009];107:65–77.
[53] Levin ED, Cerutti DT. Chapter 15: Behavioral Neuroscience of Zebrafish. In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. 2nd ed. Boca Raton(FL): CRC Press; 2009].
[54] Li N, Liu RJ, Dwyer JM, Banasr M, Lee B, Son H, et al. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biol Psychiatry 2011].
[55] Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. Nat Rev Genet 2007];8:353–67.
[56] Lopez-Patino MA, Yu L, Cabral H, Zhdanova IV. Anxiogenic effects of cocaine withdrawal in zebrafish. Physiol Behav 2008];93:160–71.
[57] Maximino C, da Silva AW, Gouveia Jr A, Herculano AM. Pharmacological analysis of zebrafish (*Danio rerio*) scototaxis. Prog Neuropsychopharmacol Biol Psychiatry 2011].
[58] Maxwell CR, Ehrlichman RS, Liang Y, Trief D, Kanes SJ, Karp J, et al. Ketamine produces lasting disruptions in encoding of sensory stimuli. J Pharmacol Exp Ther 2006];316:315–24.
[59] Moore JW, Turner DC, Corlett PR, Arana FS, Morgan HL, Absalom AR, et al. Ketamine administration in healthy volunteers reproduces aberrant agency experiences associated with schizophrenia. Cogn Neuropsychiatry 2011]:1–18.
[60] Murata S, Kawasaki K. Common and uncommon behavioural effects of antagonists for different modulatory sites in the NMDA receptor/channel complex. Eur J Pharmacol 1993];239:9–15.
[61] Myslobodsky M, Ackermann RF, Mansour R, Golovchinsky V. Ketamine-induced rotation and its interaction with naloxone in rats. Brain Res 1979];172:191–5.
[62] Myslobodsky MS, Ackermann RF, Golovchinsky V, Engel Jr J. Ketamine-induced rotation: interaction with GABA-transaminase inhibitors and picrotoxin. Pharmacol Biochem Behav 1979];11:483–6.
[63] Navarro JF, Rivera A, Maldonado E, Cavas M, de la Calle A. Anxiogenic-like activity of 3,4-methylenedioxy-methamphetamine ("Ecstasy") in the social interaction test is accompanied by an increase of c-fos expression in mice amygdala. Prog Neuropsychopharmacol Biol Psychiatry 2004];28:249–54.
[64] Neill JC, Barnes S, Cook S, Grayson B, Idris NF, McLean SL, et al. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. Pharmacol Ther 2010].
[65] NIDA. Hallucinogens and dissociative drugs, Editor's Edition, NIH, Bethesda, MD; 2010].
[66] Peng S, Zhang Y, Ren B, Zhang J, Wang H. Effect of ketamine administration on memory consolidation, p-CREB and c-fos expression in the hippocampal slices of minor rats. Mol Biol Rep 2010].
[67] Reissig CJ, Rabin RA, Winter JC, Dlugos CA. d-LSD-induced c-Fos expression occurs in a population of oligodendrocytes in rat prefrontal cortex. Eur J Pharmacol 2008];583:40–7.

[68] Ricci V, Martinotti G, Gelfo F, Tonioni F, Caltagirone C, Bria P, et al. Chronic ketamine use increases serum levels of brain-derived neurotrophic factor. Psychopharmacology (Berl) 2010].
[69] Sanchez BC, Ralston-Hooper K, Sepulveda MS. Review of recent proteomic applications in aquatic toxicology. Environ Toxicol Chem 2011];30:274–82.
[70] Silvestre JS, Nadal R, Pallares M, Ferre N. Acute effects of ketamine in the holeboard, the elevated-plus maze, and the social interaction test in Wistar rats. Depress Anxiety 1997];5:29–33.
[71] Speedie N, Gerlai R. Alarm substance induced behavioral responses in zebrafish *Danio rerio*. Behav Brain Res 2008];188:168–77.
[72] Spence R, Gerlach G, Lawrence C, Smith C. The behaviour and ecology of the zebrafish, *Danio rerio*. Biol Rev Camb Philos Soc 2008];83:13–34.
[73] Stewart A, Kadri F, DiLeo J, Chung K, Cachat J, Goodspeed J, et al. The developing utility of zebrafish in modeling neurobehavioral disorders. International Journal of Comparative Psychology 2010];23:104–21.
[74] Stewart A, Riehl R, Wong K, Green J, Cosgrove J, Vollmer K, Kyzar E, Hart P, Allain A. J. Cachat and others, Behavioral effects of MDMA ("Ecstasy") on adult zebrafish. Behav Pharmacol 2011];22(3):275–80.
[75] Stewart A, Wong K, Cachat J, Gaikwad S, Kyzar E, Wu N, Hart P, Piet V, Utterback E. M. Elegante and others, Zebrafish models to study drug abuse-related phenotypes. Rev. Neurosci. 2011];22(1):95–105.
[76] Strahle U, Grabher C. The zebrafish embryo as a model for assessing off-target drug effects. Dis Model Mech 2010];3:689–92.
[77] Swain HA, Sigstad C, Scalzo FM. Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (*Danio rerio*). Neurotoxicol Teratol 2004];26:725–9.
[78] Tang R, Dodd A, Lai D, McNabb WC, Love DR. Validation of zebrafish (*Danio rerio*) reference genes for quantitative real-time RT-PCR normalization. Acta Biochim Biophys Sin (Shanghai) 2007];39:384–90.
[79] Trompouki E, Zon LI. Small molecule screen in zebrafish and HSC expansion. Methods Mol Biol 2010];636:301–16.
[80] Van Ree JM, Leys A. Behavioral effects of morphine and phencyclidine in rats: the influence of repeated testing before and after single treatment. Eur J Pharmacol 1985];113:353–62.
[81] Verma A, Moghaddam B. NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: modulation by dopamine. J Neurosci 1996];16:373–9.
[82] Webb KJ, Norton WH, Trumbach D, Meijer AH, Ninkovic J, Topp S, et al. Zebrafish reward mutants reveal novel transcripts mediating the behavioral effects of amphetamine. Genome Biol 2009];10:R81.
[83] Westerfield M. The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*). Edition. Eugene: University of Oregon Press; 2007].
[84] White PF, Way WL, Trevor AJ. Ketamine — its pharmacology and therapeutic uses. Anesthesiology 1982];56:119–36.
[85] Williams TD, Rollins M, Block BA. Intramuscular anesthesia of bonito and Pacific mackerel with ketamine and medetomidine and reversal of anesthesia with atipamezole. J Am Vet Med Assoc 2004];225:417–21.
[86] Wilson C, Cone K, Kercher M, Hibbitts J, Fischer J, Van Lake A, et al. Naloxone increases ketamine-induced hyperactivity in the open field in female rats. Pharmacol Biochem Behav 2005];81:530–4.
[87] Wilson C, Kercher M, Quinn B, Murphy A, Fiegel C, McLaurin A. Effects of age and sex on ketamine-induced hyperactivity in rats. Physiol Behav 2007];91:202–7.
[88] Wong K, Elegante M, Bartels B, Elkhayat S, Tien D, Roy S, et al. Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). Behav Brain Res 2010];208:450–7.
[89] Wong K, Stewart A, Gilder T, Wu N, Frank K, Gaikwad S, et al. Modeling seizure-related behavioral and endocrine phenotypes in adult zebrafish. Brain Res 2010];1348: 209–15.
[90] Wright D, Krause J. Repeated measures of shoaling tendency in zebrafish (*Danio rerio*) and other small teleost fishes. Nat Protoc 2006];1:1828–31.
[91] 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];90:374–7.
[92] Xi Y, Ryan J, Noble S, Yu M, Yilbas AE, Ekker M. Impaired dopaminergic neuron development and locomotor function in zebrafish with loss of pink1 function. Eur J Neurosci 2010];31:623–33.
[93] Zakhary SM, Ayubcha D, Ansari F, Kamran K, Karim M, Leheste JR, et al. A behavioral and molecular analysis of ketamine in zebrafish. Synapse 2011];65: 160–7.
[94] Zarate Jr CA, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 2006];63:856–64.