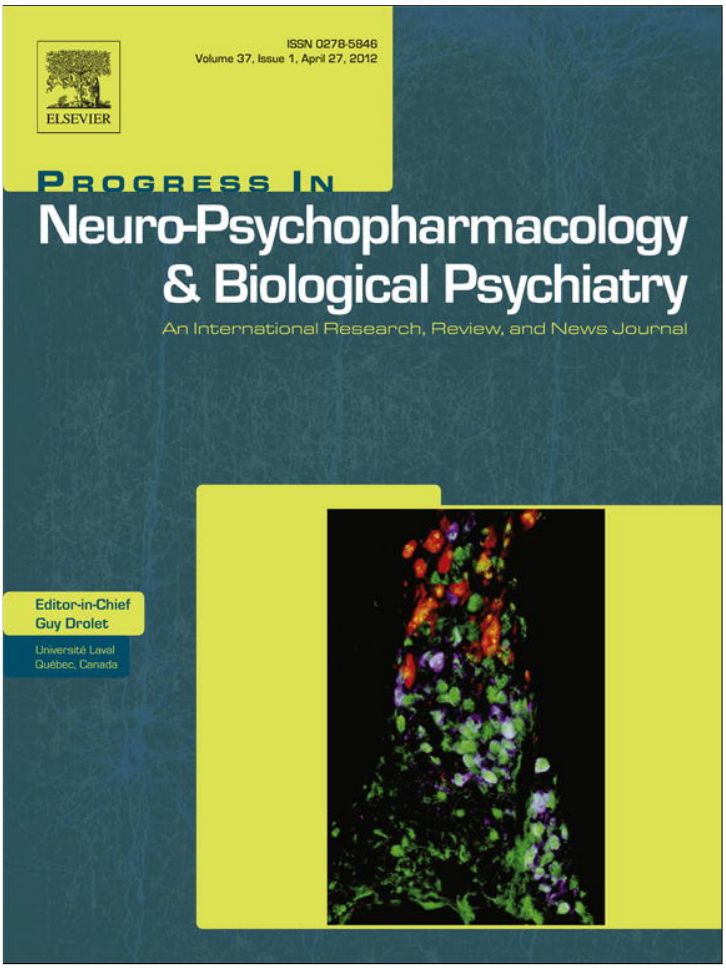

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Effects of hallucinogenic agents mescaline and phencyclidine on zebrafish behavior and physiology

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ABSTRACT

Mescaline and phencyclidine (PCP) are potent hallucinogenic agents affecting human and animal behavior. As their psychotropic effects remain poorly understood, further research is necessary to characterize phenotypes they evoke in various animal models. Zebrafish (*Danio rerio*) are rapidly emerging as a new model organism for neuroscience research. Here, we examine the effects of mescaline (5–20 mg/l) and PCP (0.5–3 mg/l) in several zebrafish paradigms, including the novel tank, open field and shoaling tests. Mescaline and PCP dose-dependently increased top activity in the novel tank test, also reducing immobility and disrupting the patterning of zebrafish swimming, as assessed by ethograms. PCP, but not mescaline, evoked circling behavior in the open field test. At the highest doses tested, mescaline markedly increased, while PCP did not affect, zebrafish shoaling behavior. Finally, 20 mg/l mescaline did not alter, and 3 mg/l PCP elevated, whole-body cortisol levels. Overall, our studies indicate high sensitivity of zebrafish models to hallucinogenic compounds with complex behavioral and physiological effects.

1. Introduction

Mescaline (3,4,5-trimethoxyphenethylamine) is one of the oldest hallucinogens known to man (Kapadia and Fayez, 1970). Produced naturally in the peyote cacti, this phenethylamine agent acts primarily on the serotonergic $5HT_{1A/2A/B/C}$ receptors (Glennon et al., 1984; Halberstadt et al., 2009; Monte et al., 1997; Palenicek et al., 2008; Shannon et al., 1984), also showing dopaminergic activity (Trulson et al., 1983). The clinical effects of mescaline, similar to the psychedelic action of lysergic acid diethylamide (LSD) and 3,4-methylenedioxymethamphetamine (MDMA), include euphoria, hallucinations, depersonalization and psychoses (Gouzoulis-Mayfrank et al., 1998; Hermle et al., 1992; Schwarz et al., 1956; Wolbach et al., 1962). In rodent models, mescaline modulates locomotion, exploration, cognitive function (Geyer et al., 1979; Koupilova et al., 1999; Palenicek et al., 2008; Sykes, 1986), aggression, startle (Palenicek et al., 2008; Sbordone and Carder, 1974; Sbordone et al., 1979) and motor responses (Canal et al., 2010; Silva and Calil, 1975; Yamamoto et al., 1992).

Phencyclidine (PCP) is another potent hallucinogenic drug, representing a synthetic arylcyclohexylamine originally developed as an anesthetic (Bristow et al., 1993; Dimpfel and Spuler, 1990; Johnson and Jones, 1990). Similar to ketamine, PCP acts as a glutamatergic *N*-methyl-D-aspartate (NMDA) antagonist (Bristow et al., 1993; Dimpfel and Spuler, 1990; Johnson and Jones, 1990), also showing cholinergic (Haring and Kloog, 1984) and monoaminergic activity (Choi et al., 2009; Nagai et al., 2009; Pozzi et al., 2010; Seeman et al., 2009). In humans, PCP evokes analgesia, ataxia, euphoria, anxiety, hallucinations and psychoses (Gorelick and Wilkins, 1989; Gorelick et al., 1986, 1989; Pearlson, 1981). Rodent models demonstrate similar behavioral effects of PCP, including hyperlocomotion, stereotypies (Sturgeon et al., 1982), context-specific alteration of anxiety (Turgeon et al., 2011) and learning/memory deficits (Willetts et al., 1990).

Mounting evidence indicates the growing significance of hallucinogenic drugs in biopsychiatry research (Check, 2004; Friedman, 2006; Halpern, 1996; Sessa, 2008). The resurgence of interest in psychedelic research requires new approaches and novel experimental models to study hallucinogenic drugs (Marona-Lewicka et al., 2011; Vollenweider and Kometer, 2010). Complementing rodent models, aquatic species can be effectively used to study these compounds (Abramson et al., 1979; Braida et al., 2007; Gettner et al., 1965; Saxena et al., 1962; Webb and Farquharson, 1971).

Abbreviations: ANOVA, analysis of variance; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; Fps, frames per second; HPI, Hypothalamic–pituitary–interrenal; LSD, lysergic acid diethylamide; MDMA, 3,4-methylenedioxymethamphetamine; NMDA, N-methyl-D-aspartate; PBS, phosphate buffered saline; PCP, phencyclidine; SPSS, statistical package for the social sciences; 5HT, serotonin.

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Zebrafish (*Danio rerio*) possess a complex behavioral repertoire, as well as fully characterized genome, and are rapidly becoming a popular model in biomedical research (He et al., 2006; Hogan et al., 2008). As a vertebrate species, they exhibit substantial physiological and morphological homology to humans, including the expression of all major brain structures, neurotransmitters, receptors, and hormones (Alsop and Vijayan, 2009; Panula et al., 2006). Adult zebrafish have already been established as a model sensitive to various hallucinogens, including both serotonergic (LSD, MDMA; Grossman et al., 2010; Stewart et al., 2011a) and glutamatergic (ketamine; Riehl et al., 2011; Zakhary et al., 2011) agents. Based on the developing utility of zebrafish for neurobehavioral research, and recent advances in video-tracking and neuroendocrine assays (Cachat et al., 2011; Egan et al., 2009), the present study aimed to characterize the effects of mescaline and PCP on zebrafish behavior and physiology.

2. Methods

2.1. Animals and housing

A total of 267 adult (5–8 month-old) "wild type" short fin zebrafish (~50:50 male:female ratio) were obtained from a commercial distributor (50 Fathoms, Metairie, LA). All fish were given at least 14 days to acclimate to the laboratory environment and housed in groups of 20–30 fish per 40-l tank. Tanks were filled with filtered system water and maintained at 25–27 °C. Illumination (1000–1100 lx) was provided by ceiling-mounted fluorescent lights on a 12-h cycle (on: 6.00 h, off: 18.00 h) according to the standards of zebrafish care (Westerfield, 2007). All fish used in this study were experimentally naïve and fed Tetramin Tropical Flakes (Tetra USA, Blacksburg, VA) twice a day. Following behavioral testing, the animals were euthanized in 500 mg/l Tricaine (Sigma-Aldrich, St. Louis, MO) and dissected on ice for further analysis.

2.2. Behavioral testing

Behavioral testing was performed between 11.00 h and 15.00 h using tanks with water adjusted to the holding room temperature. The present study used several different behavioral tests, including the novel tank, open field, and shoaling tests, as described in Grossman et al. (2010). To avoid the test battery effect, each test was performed on a separate cohort of naïve fish. Prior to testing, fish were pre-exposed in a 1-l plastic beaker for 20 min to either drug-treated or drug-free vehicle solution. During testing, zebrafish behavior was recorded by trained observers, manually scoring different behavioral endpoints (inter-rater reliability >0.85) with subsequent automated analysis of traces by Ethovision XT7 software (Noldus IT, Wageningen, Netherlands).

The novel tank test, used to assess zebrafish anxiety and locomotion (Cachat et al., 2010; Levin et al., 2007; Stewart et al., 2011a, 2011c), was a 1.5-l trapezoidal tank (15 cm height × 28 cm top × 23 cm bottom × 7 cm width; Aquatic Habitats, Apopka, FL) maximally filled with water and divided into two equal virtual horizontal portions, by a line marking the outside walls. In Experiment 1, fish were individually pre-exposed to mescaline (n = 20) or PCP (n = 13) for 20 min (see details further), and tested in the standard 6-min novel tank test. Zebrafish behavior was recorded by trained observers, scoring the latency to reach the top half of the tank (s), time spent in top (s), number of transitions to top, as well as the number and duration (s) of freezing bouts. Freezing was defined as a total absence of movement, except for the gills and eyes, for >2 s. Trials were also recorded to a computer using a USB webcam (2.0-Megapixel, Gigaware, UK) and subsequently analyzed by Ethovision XT7, assessing distance traveled (m), velocity (m/s), high mobility and immobility duration (s) (Grossman et al., 2010). Ethograms of zebrafish behavior in this test were also constructed by manually scoring episodes of bottom swimming, top swimming, bottom freezing and erratic movements, in order to visualize the occurrence of behaviors and the transitions between them. Ethograms were generated for both drug-treated and control fish, with the diameter of each circle reflecting the frequency of the behavioral activity, and the width and direction of each arrow representing the frequency of transitions between behaviors (Grossman et al., 2010).

The open field test (Experiment 2) consisted of a white plastic cylinder (21 cm diameter, 24 cm height) filled with water to a height of 12 cm. Following drug pre-treatment, the animals (n = 12–14 in each group) were individually placed in the center of the tank, and video-recorded from the top for 6 min, using Ethovision XT7 to calculate the distance traveled (m), average velocity (m/s), meandering (°/m), moving duration (s), highly mobile duration (s) and immobile duration (s), as defined in (Cachat et al., 2011). Since several psychotropic drugs, including NMDA antagonists MK-801 (Ali et al., 1994; Burket et al., 2010; Swain et al., 2004) and ketamine (Becker et al., 2003; Byrd, 1982; Riehl et al., 2011; Zakhary et al., 2011), induce prominent circling behavior in both rodent and zebrafish open field tests, we have examined the ability of mescaline and PCP to evoke rotations in our study. Video-tracking data generated by Noldus Ethovision XT7 was replayed in slow motion (~0.5 fps) and manually analyzed by two trained observers to assess zebrafish circling behavior. Rotation was defined as a full 360° circle of 5 cm (~2 fish lengths) or less in diameter, and scored as the number of left, right and total (left + right) rotations (Riehl et al., 2011). In addition, we quantified left:right rotation ratios and the number (%) of fish demonstrating 'high rotation' behavior, defined as 5 or more full rotations per a 6-min trial (Riehl et al., 2011).

The shoaling test (Experiment 3), assessing the effects of drugs on social/group behavior, was chosen based on modulation of rodent social behavior by mescaline (Poshivalov, 1980) and PCP (Sams-Dodd, 1997; Savage et al., 2011; Snigdha and Neill, 2008), and the sensitivity of zebrafish shoaling to various psychotropic drugs (Grossman et al., 2010; Riehl et al., 2011; Saverino and Gerlai, 2008; Speedie and Gerlai, 2008). Three groups of 8 zebrafish were pre-exposed in a 1-l plastic beaker for 20 min to either drug-treated water or drug-free water, and group-tested (8 fish per trial) 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. Total 24 screenshots (8 per each 8-fish shoal) per drug were used for analyses in this study, similar to (Grossman et al., 2010; Pham et al., 2012). Each screenshot was calibrated to the size of the tank and analyzed by trained observers, measuring the distances (cm) between each fish in the group using ImageTool software (University of Texas Health Sciences Center, San Antonio, TX), averaging this data to obtain an average inter-fish distance per screenshot. The number of fish in top (vs. bottom), the distance to the nearest and to the farthest neighbors were measured for each fish, and averaged over all screenshots per group (final data represented averaged results for 24 screenshots per a 24-fish cohort), similar to (Grossman et al., 2010).

2.3. Video-tracking and track reconstruction

Recorded videos were analyzed with Ethovision XT7 software, as described previously (Cachat et al., 2011). All arenas were calibrated across the bottom wall of the tanks, and the calibration axes were placed to designate the origin (0,0) at the tank center. Behavioral data were exported to Excel to generate total and per-minute plots for each endpoint. The exported traces were independently rated from 1 to n (based on similarity to each other) by three 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.

2.4. Pharmacological manipulations

Based on our previous studies with LSD, MDMA (Grossman et al., 2010; Stewart et al., 2011a) and ketamine (Riehl et al., 2011), similar in action to the serotonergic agonist mescaline and glutamatergic antagonist PCP, respectively, relatively wide dose ranges (mescaline: 5, 10 and 20 mg/l; PCP: 0.5, 1 and 3 mg/l) were selected for testing in the novel tank test (Experiment 1). This paradigm was chosen for the initial screening for its high construct, face and predictive validity, and as one of the most sensitive and commonly used zebrafish behavioral tests (Cachat et al., 2011; Egan et al., 2009; Levin et al., 2007). Since the pilot assays have established highest doses as most effective in this test, subsequent behavioral screening in the open field and shoaling tests was conducted using only 20 mg/l mescaline and 3 mg/l PCP (Experiments 2–3). To ensure drug solubility, each agent was dissolved in 0.1% vol/vol dimethyl sulfoxide (DMSO), which does not affect zebrafish swimming alone, based on published literature (Sackerman et al., 2010), and our own systematic observations (accordingly, control zebrafish were also exposed to 0.1% vehicle). The 20 min pre-treatment time was chosen given the time course of mescaline effects (Sbordone et al., 1979) and PCP (Sams-Dodd, 1995) in rodents, and based on our prior experience screening various psychotropic compounds in zebrafish models (Grossman et al., 2010; Riehl et al., 2011; Stewart et al., 2011a).

2.5. Whole-body cortisol assay

Whole-body samples for this study were taken from fish used in Experiments 1 and 3. Only 16 fish per group were used from Experiment 3. Individual body samples obtained from experimental and control cohorts were homogenized in 500 µl of ice-cold 1× PBS (phosphate buffered saline), as described in (Cachat et al., 2010). The homogenizing rotor blade was 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, Pittsburgh, PA). After ether evaporation, cortisol was reconstituted in 1 ml of 1×PBS. To quantify cortisol concentrations, ELISA was performed using a human salivary cortisol assay kit (Salimetrics LLC, State College, PA) (Cachat et al., 2010). 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, as described in Cachat et al. (2010).

2.6. Statistical analysis

The novel tank test data (Experiment 1) was analyzed in SPSS for each drug using one-way ANOVA (factor: dose), followed by a post-hoc Tukey test for significant ANOVA results. The open field and shoaling test data (Experiments 2–3) were analyzed using one-way ANOVA (factor: drug) followed by a post-hoc Tukey test for significant ANOVA results. The Mann–Whitney *U*-test was used to compare drug-treated groups with their respective controls in the ethograms and cortisol assays. Data were expressed as mean ± SEM. Significance was set at $p<0.05$ in all experiments.

3. Results

In the novel tank test (Experiment 1), mescaline significantly altered the latency to top ($F_{(3,76)}=11.9$, $p<0.0005$), transitions to top ($F_{(3,76)}=5.8$, $p<0.001$) and time in top ($F_{(3,76)}=3.8$, $p<0.05$). While the latency to top was decreased in fish treated with 10 or 20 mg/l, transitions to top and time in top were significantly elevated by 20 mg/l mescaline, with no overt effects on freezing activity, distance traveled, velocity and high/low mobility duration (Fig. 1A).

PCP significantly affected the latency to top ($F_{(3,48)}=5.0$, $p<0.005$), erratic movements ($F_{(3,48)}=5.6$, $p<0.005$) and freezing bouts ($F_{(3,48)}=2.8$, $p<0.05$) in the novel tank test. While 1 and 3 mg/l PCP reduced latency to top, 3 mg/l increased erratic movements, and 1 mg/l significantly decreased freezing bouts (Fig. 1B). PCP had no significant effects on transitions to top, time in top, freezing duration, distance traveled and velocity. Ethovision XT7-based analysis also revealed significant effects of PCP on highly mobile duration ($F_{(3,48)}=4.8$, $p<0.005$) and immobile duration ($F_{(3,48)}=2.9$, $p<0.05$), with the 3 mg/l dose reducing immobility duration and increasing highly mobile duration (Fig. 1B).

Representative traces generated by Ethovision XT7 software generally support these observations (Fig. 1). Analyzing ethograms of zebrafish novel tank behavior, we also found that both agents altered behavioral patterning of fish swimming. Mescaline (20 mg/l) increased top swimming episodes, transitions from bottom to top swimming, and transitions from top swimming to erratic movements, whereas PCP (3 mg/l) reduced bottom swimming episodes and transitions from bottom freezing to bottom swimming (Fig. 1C).

Experiment 2 assessed zebrafish behavior in the open field test, where many behavioral endpoints remained unaltered by 20 mg/l mescaline (Table 1, Fig. 2). PCP (3 mg/l) also had no effect on these endpoints, but evoked prominent circling behavior, with significantly higher left, right and total rotations, as well as more animals with high rotation phenotype in the 3 mg/l PCP fish vs. both the control and mescaline-treated groups (Table 1, Fig. 2). Representative traces, showing robust circling in PCP-treated fish, strongly support these observations (Fig. 2), with no preference of left vs. right rotations (Table 1).

Experiment 3 assessed the effects of mescaline and PCP on zebrafish shoaling behavior. Mescaline significantly affected average inter-fish distance ($F_{(2,61)}=11.1$, $p<0.0005$), distance to nearest neighbor ($F_{(2,61)}=5.2$, $p<0.01$) and distance to farthest neighbor ($F_{(2,61)}=13.1$, $p<0.0005$). As shown in Fig. 3, all three endpoints were decreased by 20 mg/l mescaline. While the control shoals were fairly tight, mescaline caused fish to remain markedly closer together while swimming in a group. In contrast, PCP (3 mg/l) had no effect on any of the endpoints in this test (Fig. 3).

In the novel tank test, whole-body cortisol levels did not significantly differ in mescaline-treated fish (4.1 ± 1 pg/g, 20 mg/l) vs. control (4.3 ± 1 pg/g, NS). In the shoaling test, cortisol levels were 4.8 ± 1 and 8.8 ± 2 pg/g, respectively (NS). In contrast, PCP (3 mg/l) significantly elevated cortisol in the novel tank test (8.2 ± 1 vs. 4.3 ± 1 pg/g in controls, $p<0.01$, *U*-test), with a similar effect in the shoaling test (24 ± 1 vs. 0.5 ± 1 pg/g, respectively, $p<0.0001$, *U*-test).

4. Discussion

As zebrafish are rapidly becoming popular animal models for neuropsychiatric disorders (Airhart et al., 2007; Baraban et al., 2007; Bencan et al., 2009; Chakraborty et al., 2009; Gonzalez-Nunez and Rodriguez, 2009), studies of hallucinogenic drug action may benefit from utilizing novel fish-based paradigms (Grossman et al., 2010; Stewart et al., 2011a). Although the hallucinogenic effects of mescaline and PCP have been studied in several species (Aanonsen and Wilcox, 1987; Audet et al., 2006; Hardman et al., 1973; Jentsch et al., 1998; Palenicek et al., 2008; Savage et al., 2011; Sbordone et al., 1979), little is known about the effects of these drugs in aquatic models. This study is the first report assessing the effects of mescaline and PCP on zebrafish behavior and physiology.

Overall, mescaline dose-dependently increased top activity and reduced immobility in the novel tank test (Fig. 1A), strikingly resembling the effects of LSD (Grossman et al., 2010), MDMA (Stewart et al., 2011a) and ketamine (Riehl et al., 2011) in zebrafish. While early reports showed hyperactivity in mice treated with mescaline (Shah, 1973, 1976), mescaline produced both anxiolytic and hyperactivating effects in zebrafish (Figs. 1–2). The anxiolytic-like *top swimming* profile evoked by mescaline in the novel tank (Fig. 1A) is consistent with

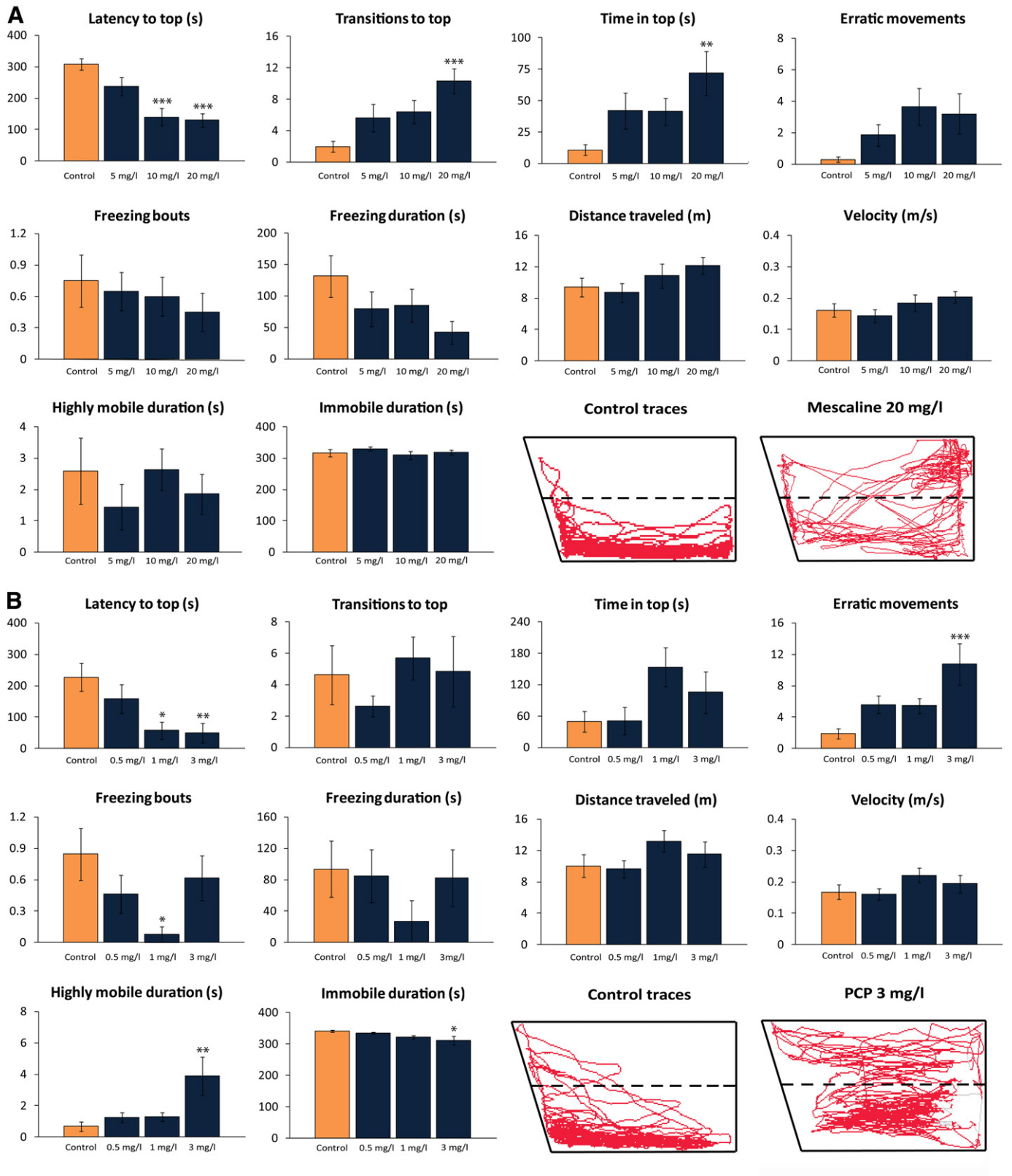

Fig. 1. Behavioral effects of 20-min acute exposure to mescaline and phencyclidine (PCP) on zebrafish tested in the novel tank (Experiment 1). Behavioral endpoints were obtained in the standard 6-min novel tank test for 5–20 mg/l mescaline (A; n = 20 per group) and 0.5–3 mg/l PCP (B; n = 13 per group). Representative 2D traces were generated by Noldus Ethovision XT7 software using the side view video-recording (the traces were examined for each experimental cohort, rated from 1 to n based on similarity, and the middle trace was selected as representative, to illustrate the patterns of zebrafish locomotion). Panel C shows the effects of drugs on the patterning of zebrafish novel tank test behavior, assessed by ethograms 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). *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. control; post-hoc Tukey test for significant ANOVA data. BS - bottom swimming, E - erratic movements, F - freezing, TS - top swimming episodes.

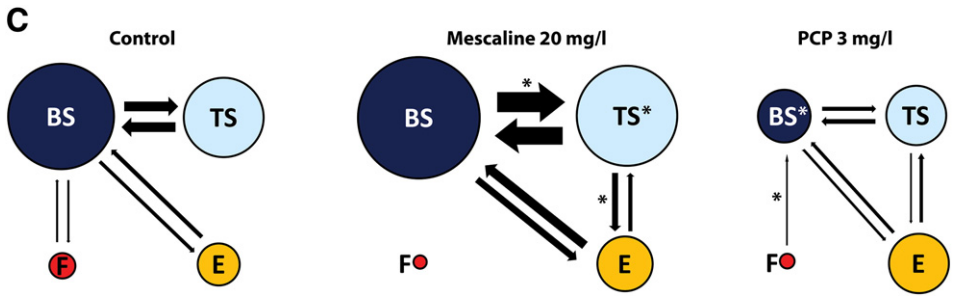

Fig. 1 (*continued*).

its ability to decrease emotionality in mice (Lush, 1975) and increase top swimming in goldfish (Abramson et al., 1979). The drug also affected the patterning of zebrafish swimming (Fig. 1C), in line with its ability to disrupt exploration in rodents (Gorelick and Bridger, 1975), which may be part of its hallucinogenic effects. PCP also evoked top dwelling, decreased latency to top and increased erratic movements in the novel tank test (Fig. 1B). Taken together, this suggests that PCP evokes hyperactivity in zebrafish, paralleling recent rodent data (McLean et al., 2010; Turgeon et al., 2011). Although shorter latency to top is generally considered anxiolytic-like in zebrafish, the parallel elevation in erratic movements suggests increased mobility.

While mescaline increases behavioral activity in rodents (Lush, 1975; Nakama et al., 1972; Shah, 1973, 1976), we found no difference in distance traveled or velocity between control and mescaline-treated fish in the open field test. PCP also did not affect open field behavior, in agreement with past rodent studies at similar doses (Lee et al., 2005; McLean et al., 2010). However, the 3 mg/l PCP group displayed longer 'highly mobile' duration in the novel tank test, paralleling manual data showing increased erratic movements and decreased immobility duration (also see ethograms in Fig. 1C). Taken together, this profile is in line with the complex effects of PCP on activity in various animal (Boren and Consroe, 1981; Byrd, 1982; Castellani and Adams, 1981; Sturgeon et al., 1979) and clinical (Domino et al., 1982; Pradhan, 1984) studies.

Prominent circling behavior induced by PCP (Fig. 2) is consistent with rotations reported in zebrafish exposed to ketamine (Riehl et al., 2011; Zakhary et al., 2011) and MK-801 (Chen et al., 2010; Seibt et al., 2010; Swain et al., 2004), and in rodents injected with PCP and other NMDA antagonists (Ali et al., 1994, 1995; Burket et al., 2010; Eshel and Korczyn, 1985; Haggerty et al., 1984; Van Ree and Leys, 1985). No overt left or right preference was found in the present study (Table 1), in agreement with our earlier zebrafish data on ketamine (Riehl et al., 2011). Interestingly, NMDA antagonists ketamine (Becker et al., 2003) and PCP (Neill et al., 2010) both trigger clinical and experimental psychoses, raising the possibility that zebrafish circling mimics psychosis-like states, or is due to the known hallucinogenic profile of PCP. However, since other hallucinogenic drugs (e.g., LSD (Grossman et al., 2010), MDMA (Stewart et al., 2011a), salvinorin A (Braida et al., 2007) and mescaline; Fig. 2) did not evoke circling in zebrafish, PCP-induced rotations observed here are likely to be associated with NMDA antagonism. The reversal of glutamatergically-induced circling by antipsychotic drugs (Seibt et al., 2010) suggests a potential pro-psychotic nature of this behavior in zebrafish, which may also contribute to effects of PCP observed here (Fig. 2).

As shown in Fig. 3, mescaline strongly affects zebrafish shoaling behavior, representing one of the most robust effects observed in this lab after screening dozens of psychotropic compounds over the last several years. While increased shoaling may be due to fear-like shoal cohesion or increased sociability, the lack of anxiogenic effects of mescaline in other tests (discussed above) implicates higher sociability, rather than anxiety-like states, in this response. Our results on increased aggregation of zebrafish support the notion that social behaviors are modulated by mescaline, exerting robust but poorly understood effects (e.g., increasing, Sbordone and Carder, 1974; Sbordone et al., 1978, 1979; or reducing aggression in animals, Poshivalov, 1980; Rewerski et al., 1971). However, all these studies agree that mescaline is a potent modulator of social behavior, and our results support this conclusion. It is also likely that mescaline distorts normal perception of other animals, thereby leading to various forms of aberrant social behaviors (Elliott and Sbordone, 1982; Poshivalov, 1980) — a factor that may have contributed to increased zebrafish shoaling (Fig. 3). Earlier reports on reduced aggression in Siamese fighting fish (Saxena et al., 1962) further support the possibility of altered social behavior by mescaline in fish (Fig. 3C). In contrast, while PCP can disrupt social behavior in several species (Brigman et al., 2009; Cleary et al., 1981; Newman et al., 2007; Russell et al., 1984; Sams-Dodd, 1996; Wilmot et al., 1987), its behaviorally active dose (3 mg/l) did not affect zebrafish shoaling (Fig. 3), in line with some rodent studies showing no PCP effects on social/aggressive behavior (Miczek and Haney, 1994; Tyler and Miczek, 1982). Since we only explored the effects of acute drug exposure, further studies using longer-term and/or repeated treatment, as well as applying various agonists and antagonists, may provide further insights into the social effects of mescaline and PCP in zebrafish.

The search for novel biomarkers is becoming an important direction in drug abuse research. Endocrine phenotypes, such as cortisol released by the hypothalamic–pituitary–interrenal (HPI) system may represent an interesting aspect to assess. Analyzing whole-body cortisol data, we noted an increase produced by PCP (3 mg/l) in both the novel tank and shoaling tests. This profile is consistent with the possibility of hallucinogenic/psychotogenic action (Gorelick and Balster, 1995), since such states are commonly associated with elevated

Table 1
Behavioral effects of 20-min acute exposure to mescaline and phencyclidine (PCP) on zebrafish tested in the open field test (Experiment 2). Behavioral data were generated by Noldus Ethovision XT7 software using the top view video-recording. Rotational data (also see Fig. 2 for details) were obtained during manual observation of the recorded videos, using a slow-mode replay function. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. control, &$p<0.05$ vs. mescaline-treated group ($n=12$–14 per group); post-hoc Tukey test for significant ANOVA data; NS — no significant difference by ANOVA.

Endpoints	Control	Mescaline 20 mg/l	PCP 3 mg/l	ANOVA data
Distance traveled (m)	15 ± 1.3	11 ± 1.5	12 ± 1.3	$F_{(2,37)}=2.3$, NS
Velocity (m/s)	0.26 ± 0.02	0.19 ± 0.03	0.25 ± 0.03	$F_{(2,37)}=2.1$, NS
Moving duration (s)	282 ± 12	204 ± 29*	253 ± 16	$F_{(2,37)}=3.4$, $p<0.05$
Highly mobile duration (s)	4.4 ± 0.9	3.5 ± 0.7	7.2 ± 3.7	$F_{(2,37)}=0.8$, NS
Immobile duration (s)	223 ± 14	261 ± 18	236 ± 12	$F_{(2,37)}=1.4$, NS
Meandering (1000°/m)	425 ± 153	1370 ± 434	571 ± 129	$F_{(2,37)}=3.2$, NS
Left rotations	2 ± 0.9	1.1 ± 0.5	5.8 ± 1.5*&	$F_{(2,38)}=5.4$, $p<0.01$
Right rotations	1.2 ± 0.4	3.1 ± 1.2	8.9 ± 2.1**&	$F_{(2,38)}=7.8$, $p<0.001$
Total rotations	2.5 ± 0.8	4.2 ± 1.6	15 ± 3.4***&	$F_{(2,38)}=8.6$, $p<0.001$

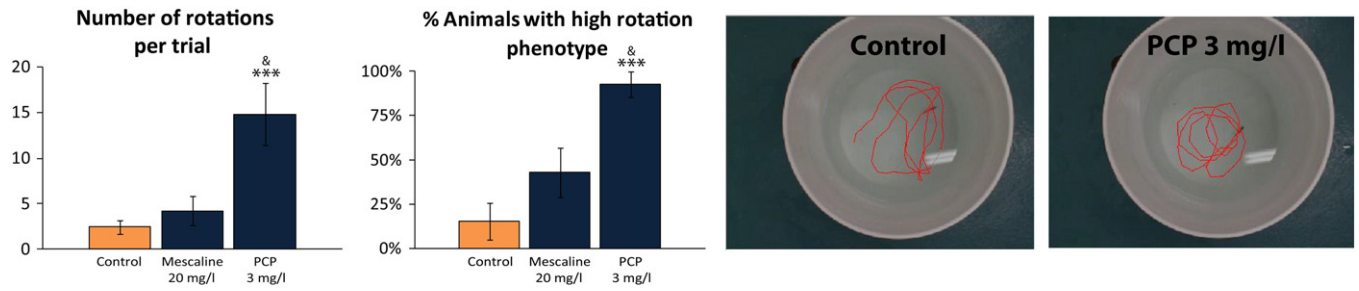

Fig. 2. Behavioral effects of 20-min acute exposure to mescaline and phencyclidine (PCP) on zebrafish rotational behavior in the open field test (Experiment 2). Rotation data represent the number of total (left + right) rotations and the percentage of animals per group displaying 'high rotation' behaviors (5 or more 'full' rotations) during the 6-min test ($n = 13–14$ per group). ANOVA results show a significant drug effect on the number of total rotations ($F_{(2,38)} = 8.6$, $p<0.001$) and the percentage of animals showing 'high rotation' phenotype ($F_{(2,38)} = 13.2$, $p<0.001$). Representative traces showing rotation behavior in PCP-treated fish (vs. control) were generated by Noldus Ethovision XT7 software using the top view video-recording (only a 1-min segment of a 6-min test is presented for clarity). 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 (see Table 1 for details of other behaviors observed in the open field test). ***$p<0.001$ vs. control, &$p<0.05$ vs. mescaline group; post-hoc Tukey test for significant ANOVA data.

glucocorticoids in non-human primates (Elvidge et al., 1976; Setchell et al., 1975) and rodents (Deutsch et al., 1983; Pechnick et al., 1986, 1989) following PCP administration. Interestingly, a related compound, ketamine, lowered cortisol levels in zebrafish (Riehl et al., 2011), which may be due to varying psychopharmacological profiles, with ketamine exerting anxiolytic-like clinical and preclinical effects (Annetta et al., 2005; Engin et al., 2009), and PCP showing much less robust responses (Gonzalez-Maeso and Sealfon, 2009; Gorelick and Balster, 1995). In contrast, the behaviorally active dose of mescaline (20 mg/l) did not significantly alter cortisol in the novel tank and shoaling tests. Although relatively little is known about the effect of mescaline on glucocorticoids (Check, 2004; Sackler et al., 1971), the lack of cortisol elevation observed in this study may be due to a combination of the anxiolytic and psychotomimetic activities of mescaline. While LSD increased cortisol levels in zebrafish (Grossman et al., 2010), another serotonergic hallucinogen, MDMA, did not produce consistent effects on cortisol (own unpublished observations), indirectly supporting this notion. Clearly, further studies are needed to assess endocrine correlates of various hallucinogenic drugs in zebrafish. There were several other limitations of this study. First, it did not include repeated or chronic treatment, which may further elucidate some of the complex neural effects of mescaline and PCP (Neill et al., 2010; Poshivalov, 1980). Also, drug administration via other routes (vs. immersion), and measuring the drug concentrations in the brain, may be necessary.

Finally, comparison of mescaline and PCP with other hallucinogenic drugs reveals interesting parallels between their relative efficacies. Since psychedelic doses in humans are approximately 200 mg for

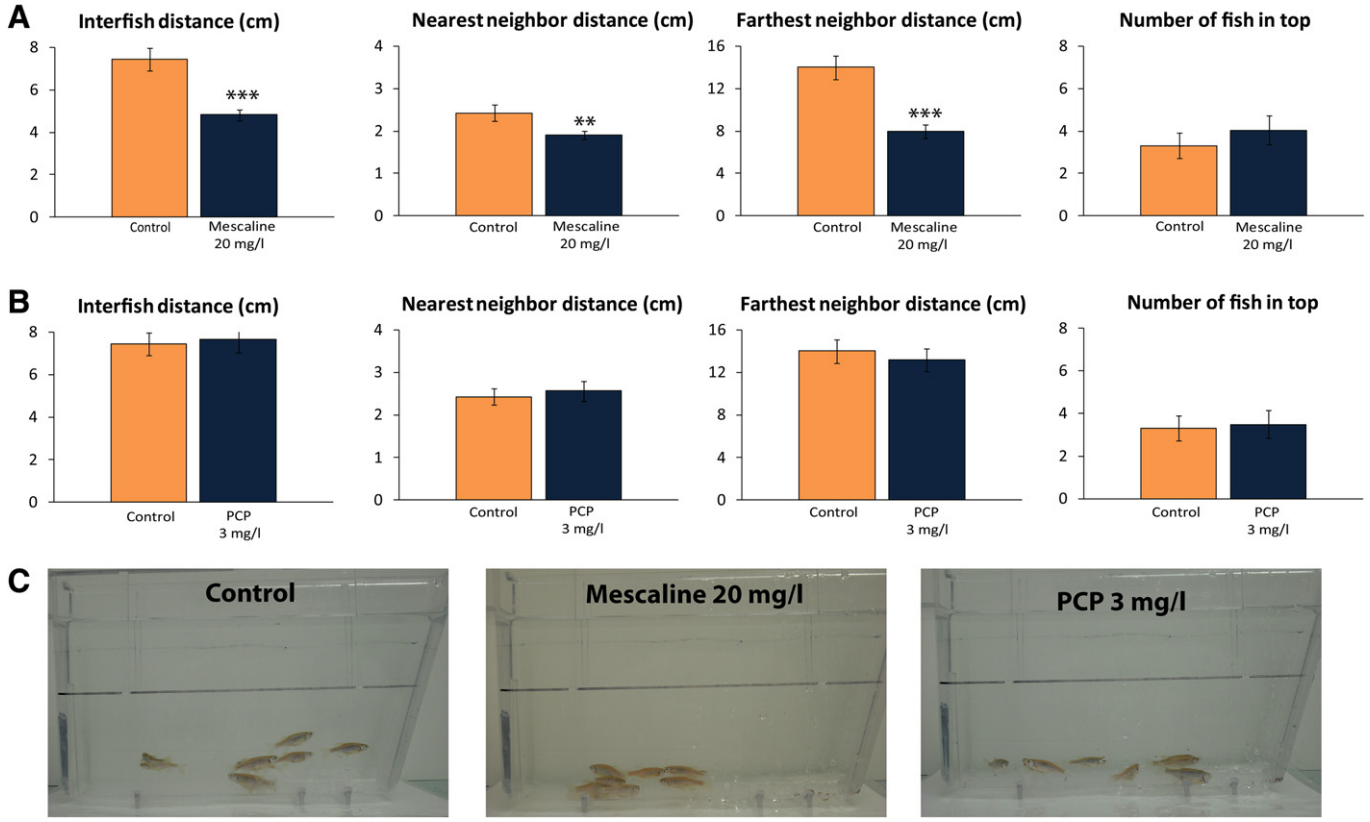

Fig. 3. Behavioral effects of 20-min acute exposure to mescaline (A) and phencyclidine (PCP; B) on zebrafish tested in the shoaling tests (Experiment 3). Panel C shows typical patterns of zebrafish shoaling evoked by these drugs (representative photographs for each cohort were rated from 1 to n, based on similarity to each other, and the middle image was selected as representative, to illustrate the patterns of zebrafish shoaling). **$p<0.01$, ***$p<0.001$ vs. control ($n = 24$ per group); post-hoc Tukey test for significant ANOVA data.

mescaline and MDMA, >10 mg for PCP, 125 mg for ketamine and <1 mg for LSD, mescaline is equally potent as MDMA, >200-fold less potent than LSD, 10–20 fold less potent than PCP, and twice less potent than ketamine. In zebrafish, the effective dose of mescaline (20 mg/l) was 15 times less potent than PCP (3 mg/l; Fig. 1), 200-fold less potent than LSD (0.1 mg/l; Stewart et al., 2011b), 4 times less potent than MDMA (80 mg/l; Stewart et al., 2011a), and twice less potent than ketamine (40 mg/l; Riehl et al., 2011). Human 'psychedelic' doses of PCP (>10 mg) are ~20 times stronger than mescaline and MDMA, 10–30 times less potent than LSD, and ~10 times more potent than ketamine. In the present study, the dose of PCP (3 mg/l) active in zebrafish was ~30-fold weaker than LSD (0.1 mg/l; Stewart et al., 2011b), ~30-fold stronger than MDMA (80 mg/l; Stewart et al., 2011a), and ~10-fold stronger than ketamine (40 mg/l; Riehl et al., 2011). Collectively, this shows that relative efficacy of mescaline, PCP and other common hallucinogens in fish strikingly resemble that of humans, further supporting the growing translational value of zebrafish models for psychedelic drug research.

5. Conclusion

Overall, zebrafish paradigms are highly sensitive to various drugs of abuse (Bencan et al., 2009; Fernandes and Gerlai, 2009; Gerlai et al., 2006, 2008; Levin et al., 2007), providing a useful animal model to study hallucinogenic drugs, which will likely increase our understanding of the neurobiology of drug-induced neurobehavioral disorders. In line with this, the behavioral and physiological changes elicited in adult zebrafish by mescaline and PCP (Figs. 1–3) parallel drug-evoked responses observed in clinical patients and other animal models.

The similarity of effects evoked in zebrafish by two psychedelic drugs (Fig. 1) sharing some clinical and pre-clinical hallucinogenic effects, but acting via different pharmacological mechanisms, raises another important question: Can aquatic zebrafish-based tests serve as a useful specific screen for testing various hallucinogenic drugs? Recent zebrafish data on LSD, MDMA, ketamine (Grossman et al., 2010; Riehl et al., 2011; Stewart et al., 2011a) and our present findings with mescaline and PCP (Figs. 1–3) seem to support this notion. The possibility of developing such high-throughput zebrafish-based screens not only offers an evolutionary perspective on drug-induced states, but also fosters further searches for new compounds with potential pro- and anti-hallucinogenic properties.

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