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Acute exposure to waterborne psychoactive drugs attract zebrafish

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ABSTRACT

Psychotropic medications are widely used, and their prescription has increased worldwide, consequently increasing their presence in aquatic environments. Therefore, aquatic organisms can be exposed to psychotropic drugs that may be potentially dangerous, raising the question of whether these drugs are attractive or aversive to fish. To answer this question, adult zebrafish were tested in a chamber that allows the fish to escape or seek a lane of contaminated water. These attraction and aversion paradigms were evaluated by exposing the zebrafish to the presence of acute contamination with these compounds. The zebrafish were attracted by certain concentrations of diazepam, fluoxetine, risperidone and buspirone, which were most likely detected by olfaction, because this behavior was absent in anosmic fish. These findings suggest that despite their deleterious effects, certain psychoactive drugs attract fish.

1. Introduction

Psychotropic medications such as antidepressants, antipsychotics and anxiolytics are widely used (Bocquier et al., 2008) and its prescription has increased worldwide in the last 20 years (Carta et al., 2004; Paulose-Ram et al., 2007; Alonso et al., 2004; la Poza et al., 2013). Consequently, increasing its presence in aquatic environments (Santos et al., 2007) which are monitored especially in urban and hospital wastewater, effluent from water and sewage treatment plants, surface and drinking water (Calisto et al., 2011; Al Aukidy et al., 2012). The main concern is that these contaminants may cause toxicity, affecting the health of non-target humans and animals. Also, many of these drugs are resistant to wastewater treatments and are only partially removed (Palmer et al., 2008; Silva et al., 2011).

The most commonly prescribed, consumed, and consequently detected drugs in aquatic environments are benzodiazepines, selective serotonin reuptake inhibitors (SSRIs), buspirone, risperidone, and ethanol. Benzodiazepines, such as diazepam and clonazepam, potentiate $GABA_A$ receptor function by increasing the channel opening frequency, producing hypnotic effects by acting on the α1 subunit (McKernan et al., 2000) and anxiolytic effects by acting on the α2 subunit (Löw et al., 2000). Fluoxetine is a potent and highly selective inhibitor of the transporter for serotonin reuptake at the presynaptic membrane, causing increases in serotonin concentrations at postsynaptic receptor sites (Wong et al., 1995). Buspirone exerts anxiolytic effects by acting as a partial agonist at serotonin 5-HT1A receptors (Ohlsen and Pilowsky, 2005), and it also interacts to a lesser degree with other receptors, such as the dopamine D2 receptor (Dhavalshankh et al., 2007). The antipsychotic drug risperidone belongs to the benzisoxazole chemical class (Kumar et al., 2008; Courchesne et al., 2007) and has been reported to act therapeutically by blocking serotonin and dopamine receptors (Grant, 2007); thus, it is useful for studying increases in serotonin neurotransmission. Ethanol also has acute anxiolytic effects that are most likely mediated by $GABA_A$ receptors (Radcliffe et al., 1999; Kumar et al., 2009), with depressant effects on the central nervous system at higher doses.

Although the concentrations of these drugs in aquatic environments are lower than the lethal concentrations for most of the species present in these ecosystems, studies have shown that their concentrations in organs such as the brain, liver and muscles are higher than those in the water (Brodin et al., 2013; Brooks et al., 2005; Sackerman et al., 2010). Benzodiazepines and SSRIs may trigger a set of morphological, physiological, neuroendocrine, reproductive, motor and behavioral changes (Brodin et al., 2013; Sackerman et al., 2010; Airhart et al., 2007; Gebauer et al., 2011; Park et al., 2012; Prieto et al., 2012; Abreu et al., 2014; Idalencio et al., submitted for publication).

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Since these psychoactive drugs are potentially dangerous to fish, we posed the following question: are these drugs attractive or aversive to fish? To answer this question, adult zebrafish were placed into a chamber that allowed them to avoid or to swim into a lane containing contaminated water. This enabled the evaluation of the attraction and aversion paradigm in zebrafish exposed to acute contamination of these compounds.

2. Methods

2.1. Ethics statement

This study was approved by the Ethics Commission for Animal Use (CEUA) at the Universidade de Passo Fundo, UPF, Passo Fundo, RS, Brazil (Protocol 29/2014-CEUA) and met the guidelines of the Conselho Nacional de Controle de Experimentação Animal (CONCEA).

2.2. Subjects

A mixed-sex stock population of adult wild-type zebrafish (*Danio rerio*) from the short-fin (SF) strain was used. In the experiment 1, ten fish were subjected to each substance treatment, totalizing 210 fish (21 treatments, each with 10 fish). In the 2nd experiment, ten anosmic fish were subjected to the substances that are attractive or aversive in the 1st experiment and also a saline only control, thus, a total of 200 fish were used in this study.

The fish were fed twice per day at 10:00 and 16:00 h with a commercial flake food until satiation (Alcon® Basic, MEP 200 Complex, Brazil). The mean water temperature in the holding tank was maintained at 24 ± 2 °C, and the dissolved oxygen concentrations varied from 5.6 to 7.2 mg/l (both measured using YSI model 550A oxygen meter; Yellow Springs Instruments, USA). The pH values ranged from 6.2 to 7.4 (measured using a Bernauer pH meter). The total ammonia–nitrogen concentration was less than 0.5 mg/l (measured using a colorimetric test).

2.3. Substances

Clonazepam (Rivotril®), diazepam (União Química, Brazil), fluoxetine (Daforin, EMS), risperidone (Risperidona, EMS), buspirone (Ansitec®, LIBBS) and ethanol were purchased from common commercial suppliers. The details of the substances examined in the experiment are listed in Table 1. The food odor positive controls were prepared using two distinct methods. Positive control 1 was prepared by adding flaked food to the water at a rate of 0.5 g/l, followed by the homogenization and the immediate use of the mixture in specific test trials. Positive control 2 differed from positive control 1 only in that the flaked food remained in the water overnight (12 h) before the mixture was homogenized and used in specific test trials.

2.4. Experimental apparatus

The experimental apparatus consisted of a modified, 30-liter acrylic tank (50 × 25 × 25 cm, length × width × height). Metal mesh was added to prevent the fish from escaping the tank. A short segregation panel and a fine mesh baffle were inserted at the other end of the tank to create two chambers leading to two lanes of water with laminar flow run in parallel without mixing. See the schematic drawing of the apparatus in Fig. 1A and the dye (gentian violet) colored confirmation of laminar flux for all substances in Fig. 1B. The use of the dye aimed to verify if the separate flux was maintained in all drug tests, and drugs were not mixed to the dye during the experiments. A flow rate of 2 l/min was used for each track, and the manifold for each mixing chamber had a single door to allow for the introduction of the test substance.

2.5. Experimental protocol

In experiment 1, individual fish were transferred from the holding tank in a small volume of water. After transfer, the fish were allowed to acclimate for 150 s, and a continuous dose of the test compound was subsequently introduced into one of the mixing chambers for 150 s at a predetermined concentration. During the tests, fish were not fed. The position (left or right) of the clean and contaminated water lanes was switched between each of the trials to prevent a possible bias caused by a fish preference for the left or right lane. The horizontal gradient created by the laminar flow within the tank allowed for the untreated lane to remain uncontaminated, thus creating two lanes between which the fish could move freely (Readman et al., 2013). Following each single fish testing, the system was manually flushed to remove any test substance residue. The location and locomotor activity of the fish with access to both the treated and untreated lanes were recorded with a video camera for the entire experimental period. The video camera was positioned directly above the tank. The analysis of the video recordings was conducted using ANY-maze® video tracking system (Stoelting Co., USA) for both the 150-s acclimation period and the 150-s exposure period to show that the fish responded only after substance introduction, and the results for each test substance were analyzed separately.

The experiment 2 reproduces the 1st one but using zebrafish with temporary anosmia by the application of lidocaine gel (50 mg/g) in the nares and olfactory surface as described by Johansen (Johansen,

Table 1
Effects of substances and concentrations.

Substance	Concentration	Effect	Reference
Water (control)	–	–	–
pH 3 (Trichloroacetic acid)	pH 3	Escape behavior	Readman et al. (2013)
Ethanol	1%	Neuroendocrine changes	Oliveira et al. (2013)
Ethanol	0.5%	Neuroendocrine changes	Oliveira et al. (2013)
Ethanol	0.25%	Neuroendocrine changes	Oliveira et al. (2013)
Clonazepam	0.057 μg/l	Ambient concentration	Almeida et al. (2013)
Clonazepam	300 μg/l	Behavior changes	Gebauer et al. (2011)
Diazepam	160 μg/l	Neuroendocrine changes	Abreu et al. (2014)
Diazepam	16 μg/l	Neuroendocrine changes	Abreu et al. (2014)
Diazepam	0.88 μg/l	Ambient concentration	Calisto and Esteves (2009)
Fluoxetine	50 μg/l	Neuroendocrine changes	Abreu et al. (2014)
Fluoxetine	25 μg/l	Neuroendocrine changes	Abreu et al. (2014)
Fluoxetine	1 μg/l	Neuroendocrine changes	Abreu et al. (2014)
Risperidone	0.00034 μg/l	Ambient concentration	Calisto and Esteves (2009)
Risperidone	100 μg/l	Behavior changes	Magno (2012)
Risperidone	170 μg/l	Neuroendocrine changes	Idalencio et al. (submitted for publication)
Buspirone	10 μg/l	Behavior changes at 1% concentration	-
Buspirone	1000 μg/l	Behavior changes	Gebauer et al. (2011)
Buspirone	3000 μg/l	Behavior changes	Gebauer et al. (2011)