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Pharmacological analysis of zebrafish (*Danio rerio*) scototaxis

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ABSTRACT

The scototaxis test has been introduced recently to assess anxiety-like phenotypes in fish, including zebrafish. Parametric analyses suggest that scototaxis represents an approach–avoidance conflict, which hints at anxiety. In this model, white avoidance represents anxiety-like behavior, while the number of shuttling events represents activity. Acute or chronic fluoxetine, buspirone, benzodiazepines, ethanol, caffeine and dizocilpine were assessed using the light–dark box (scototaxis) test in zebrafish. Acute fluoxetine treatment did not alter white avoidance, but altered locomotion in the higher dose; chronic treatment (2 weeks), on the other hand, produced an anxiolytic effect with no locomotor outcomes. The benzodiazepines produced a hormetic (inverted U-shaped) dose–response profile, with intermediate doses producing anxiolysis and no effect at higher doses; clonazepam, a high-potency benzodiazepine agonist, produced a locomotor impairment at the highest dose. Buspirone produced an anxiolytic profile, without locomotor impairments. Moclobemide did not produce behavioral effects. Ethanol also produced a hormetic profile in white avoidance, with locomotor activation in 0.5% concentration. Caffeine produced an anxiogenic profile, without locomotor effects. These results suggest that the light–dark box is sensitive to anxiolytic and anxiogenic drugs in zebrafish.

1. Introduction

Zebrafish (*Danio rerio* Hamilton 1822) are small cyprinid fishes which have long been used as models in developmental and genetic studies (Key and Devine, 2003). Its physiology is relatively simple, intermediary between humans and, e.g., flies and worms, makes it suitable for high-throughput research in pharmacology, toxicology, behavioral genetics and pharmacogenomics (Gerlai, 2010; Stewart et al., 2010). They also present neuroanatomical landmarks and neurotransmitter systems which are very similar to those observed in mammals (Maximino and Herculano, 2010; Panula et al., 2010), and respond in a predictable fashion to anxiolytic and anxiogenic drugs in behavioral screens such as the novel tank diving test (Bencan et al., 2009; Cachat et al., 2010; Egan et al., 2009) or the open-field (López-Patiño et al., 2008). In fact, recently many different behavioral tests of anxiety, fear and stress have been proposed using zebrafish (Maximino et al., 2010a).

Aside from the already mentioned novel tank diving test and open-field, the scototaxis test has also been proposed as a model of anxiety-like behavior in different teleost species (Maximino et al., 2010c; Stewart et al., 2010). Different from the novel tank diving test (Bencan et al., 2009; Egan et al., 2009; Grossman et al., 2010; Sackerman et al., 2010; Sallinen et al., 2009; Stewart et al., 2010; Stewart et al., in press a; Wong et al., 2010), in which the novelty of the environment is the main aversive stimulus (Bencan et al., 2009; Wong et al., 2010), behavior in the scototaxis test is driven mainly by a approach–avoidance motivational conflict (Maximino et al., 2010c). The test is deceptively simple, very similar to the murine light/dark box (Bourin and Hascöett, 2003), relying on the exploration, by fish, in a black and white tank for the establishment of preference (Maximino et al., 2010c; Stewart et al., 2010). In general, anxiolytic drugs and treatments increase the time the animal spends in the white compartment while anxiogenic drugs decrease this time (Grossman et al., 2010; Sackerman et al., 2010; Stewart et al., 2010).

Other models of anxiety in zebrafish (such as open-field and the novel tank diving test) have demonstrated behavioral effects of anxiolytic and anxiogenic agents (Bencan et al., 2009; Egan et al., 2009; Grossman et al., 2010; López-Patiño et al., 2008; Sackerman et al., 2010; Sallinen et al., 2009; Stewart et al., 2010; Stewart et al., in press b; Wong et al., 2010). The scototaxis test has the advantage of being more extensively validated (behaviorally) than other tasks. For example, high-avoidant animals (i.e., animals which spend less time

Abbreviations: 5-HT, 5-hydroxytryptamine,serotonin; DPCPX, 8-Cyclopentyl-1,3-dirpopylxnathine; LSD, Lysergic acid diethylamide; MAO-A, Monoamine oxidase A; SERT, Serotonin transporter; SSRI, Selective serotonin reuptake inhibitor.

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in the white compartment when first exposed to the apparatus), when confined in the white compartment, show increased freezing and erratic movement (Blaser et al., 2010), which suggest that approach to the black compartment is not what determines the preference for the dark environment in this model. High-avoidant animals also show increased thigmotaxis ("clinging" to the walls of the apparatus) in the black compartment (Blaser et al., 2010). Moreover, intra- and inter-session habituation of locomotion, but not of white avoidance, suggest that the white compartment is indeed aversive, but that a second component elicits exploration of this compartment as the session evolves (Maximino et al., 2010b). Increasing lighting levels above the white portion of the tank decreases the time spent in it during the session (Stewart et al., 2010); confining animals thrice in the white compartment prior to the experiment does not alter spatiotemporal measures of preference, but decrease the frequency of burst swimming, freezing and thigmotaxis in the white compartment, suggesting that this treatment diminishes fear (Maximino et al., 2010b). When animals are separated in high-avoidant versus low-avoidant, one single confinement event decreases the time spent in the white compartment in high-avoidant, but increases this latter measure in low-avoidant zebrafish (Blaser et al., 2010). Overall, these results suggest that scototaxis is not resultant from approach to the black compartment nor from avoidance of the white compartment, being instead the compound result of an approach–avoidance conflict; stimulus control, then, is the resultant from these conflicting motivations. This is important, since it has been suggested that, at least in rodents, novelty is not enough to produce anxiety, inducing a state more akin to arousal (Misslin and Cigrang, 1986). The choice of drugs in the present experiments reflects the objective of further analyzing scototaxis as an anxiety model.

Pharmacological analyses of this test have been few and far in between. Preliminary results from our laboratory and by Su Guo uncovered an anxiolytic effect of low doses of chlordiazepoxide (Lau et al., 2010; Maximino et al., 2010c), a compound which reduces theta frequency in the hippocampus of rats (Woodnorth and McNaughton, 2002) and produces anxiolytic effects in the light–dark transitions box in mice (Chaouloff et al., 1997; Griebel et al., 1996; Hascoet and Bourin, 1998; Shimada et al., 1995) and in the cat odor challenge model in rats (Zangrossi and File, 1992); interestingly, chlordiazepoxide was not detected as an anxiolytic compound in the novel tank diving test (Bencan et al., 2009). Caffeine is also anxiogenic in the novel tank test (Cachat et al., 2010; Egan et al., 2009) and in the scototaxis test (Stewart et al., 2010), and the A_1 adenosine receptor inverse agonist DPCPX is also anxiogenic in the scototaxis task (Stewart et al., 2010). Nicotine did not produce any significant effect on total locomotion or white avoidance in a modified version of the scototaxis test, but acute ethanol and chlordiazepoxide increased the time spent in the white arms (Sackerman et al., 2010). The acute exposure of zebrafish to acute citalopram (an selective serotonin reuptake inhibitor which binds on the allosteric site of the serotonin transporter) or yohimbine (an α-adrenoceptor antagonist, and, to a lesser extent, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, and D_2 receptor antagonist, and 5-HT_{1A} receptor partial agonist) do not produce an anxiogenic effect, though (Sackerman et al., 2010). Acute exposure to LSD also produces an anxiolytic-like effect in zebrafish (Grossman et al., 2010).

The present article extends these findings, analyzing the effects of acute and chronic treatment with fluoxetine, a selective serotonin reuptake inhibitor (SSRI) which binds to the orthosteric site of the serotonin transporter; diazepam and chlordiazepoxide, classic benzodiazepine receptor agonists; clonazepam, a high-potency benzodiazepine receptor agonist; buspirone, a 5-HT_{1A} partial agonist; moclobemide, a monoamine oxidase inhibitor; acute ethanol; and caffeine. These drugs were chosen based on their clinical effects on generalized anxiety disorder (SSRIs, classic and high-potency benzodiazepines, and buspirone) or panic disorder (SSRIs, MAOIs, and high-potency benzodiazepines) (Lieberman and Tasman, 2006). Caffeine and ethanol were chosen because they show effects in other models of anxiety in zebrafish (Cachat et al., 2010; Egan et al., 2009), and are extensively used by the population outside of clinical settings.

2. Methods

2.1. Animals and housing

240 unsexed adult wildtype zebrafish (shortfin phenotype) were kept in collective 40 l tanks ($n = 20$ fish per tank) for two weeks before experiments begun. The water was reconstituted and buffered to a pH of 7.0 (Mydor Target 7.0 buffer), and the tanks had constant filtering, temperature control (27 ± 2 °C), illumination (14/10 h, beginning of the cycle at 0700 am) and feeding (Oscar Gold pellet ration). Animals were not used for any other experiment besides the one presented in this paper. Rearing and welfare conditions were in accordance with the standards set by ASAB/ABS (2006) and Colégio de Experimentação Animal, COBEA/Brazil (Andersen et al., 2008), and were approved by UFPA's Ethics Committee.

2.2. Drug treatments

Fluoxetine hydrochloride (Eli Lily, Brazil), buspirone hydrochloride (Bristol-Myers Squibb, Brazil), moclobemide (Roche, Brazil), ethanol (Cromoline, Brazil), and anhydrous caffeine (Quimis, Brazil) were dissolved in teleost's normal Ringer solution (115 mM NaCl, 2.9 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.2) (Westerfield, 2000) in fresh preparations made 2 h before the experiment. Clonazepam (Roche, Brazil), diazepam (Roche, Brazil), and chlordiazepoxide (Farmasa, Brazil) were dissolved in a solution of 40% propylene glycol, 10% ethyl alcohol, 5% sodium benzoate, and 1.5% benzyl alcohol (Maximino et al., 2010c). Animals were injected with vehicle (teleost's Ringer solution), 5.0, or 10.0 mg kg^{-1} fluoxetine; vehicle (propylene glycol/ethyl alcohol/sodium benzoate/benzyl alcohol solution), 0.05, 0.5 or 1.0 mg kg^{-1} clonazepam; vehicle (propylene glycol/ethyl alcohol/sodium benzoate/benzyl alcohol solution), 0.02 or 0.2 mg kg^{-1} diazepam; vehicle (propylene glycol/ethyl alcohol/sodium benzoate/benzyl alcohol solution), 0.02 or 0.2 mg kg^{-1} chlordiazepoxide; vehicle (teleost's Ringer solution), 25.0 or 50.0 mg kg^{-1} buspirone; vehicle (teleost's Ringer solution), 5.0 or 10.0 mg kg^{-1} moclobemide; vehicle (teleost's Ringer solution), 0.25%, 0.5% or 1.0% (v.v.) ethanol; or vehicle (teleost's Ringer solution) or 100 mg kg^{-1} caffeine. For chronic treatment with fluoxetine, animals were injected daily, for 2 weeks, with the same doses as in the acute treatment. Before injection, animals were kept in water containing (±)menthol (100 mg l^{-1}, Aldrich, St. Louis, MO, USA) until anesthetized, and were subsequently weighted; control animals were equally handled, anesthetized and injected with teleost's Ringer solution daily for 2 weeks. The injected volume was between 4 and 6 μl, depending on the weight of the fish (0.4–0.6 g). 30 min. after drug treatment, animals were tested in the 15-min scototaxis test. Caffeine-treated animals were tested for 10 min, and not 30 min, after drug treatment, as it has been shown to produce an anxiogenic effect after 15 min, but not 30 min, in mice (Jain et al., 1995).

2.3. Apparatus and procedure

The test tank consisted of an aquarium made of matte acrylic (15 × 10 × 45 cm), with one horizontal half made of white acrylic and the other half made of black acrylic. The acrylic chosen was not reflective, in order to avoid the tendency of those animals which present shoaling to behave in relation to their own reflection. The tank contained sliding central doors, colored with the same color of the aquarium side, defining a central compartment of 15 × 10 × 10 cm.