Progress in Neuro-Psychopharmacology & Biological Psychiatry 35 (2011) 624–631

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Pharmacological analysis of zebrafish (*Danio rerio*) scototaxis

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ARTICLE INFO

Article history:
Received 27 October 2010
Received in revised form 4 January 2011
Accepted 4 January 2011
Available online 13 January 2011

Keywords:
Anxiety
Light/dark preference
Zebrafish

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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0278-5846
doi:10.1016/j.pnpbp.2011.01.006

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.

For the present experiment, we used the protocol described in Maximino et al. (2010c). Briefly, in each session animals were placed individually in the central compartment for 3 min (acclimation), after which the sliding doors were removed. The animals were then allowed to freely explore the aquarium. The session was terminated after 900 s. The proportion of the trial that the animal spent in the white compartment and the number of shuttle events were recorded. After each trial within one session, the tank was rotated by 180°, so as to eliminate spatial effects. The tank was illuminated by environmental light (60 W light bulb, located at 1.80 m above the tank top) which kept illumination uniform and constant between trials.

2.4. Statistical analyses

Data were analyzed using one-way analyses of variance (ANOVAs) followed by Dunnett's multiple comparison test when appropriate. Data from caffeine treatment were analyzed using Student's *t*-test. All analyses and figures were made using GraphPad Prism 5.00 (GraphPad Software, Inc.), and data is presented as mean ± standard error.

3. Results

Acute fluoxetine treatment did not alter white avoidance in zebrafish (Fig. 1A, $F_{[2, 29]} = 0.5236$, NS), but it increased locomotion at the highest dose (Fig. 1B, $F_{[2, 26]} = 5.441$, $p = 0.0113$). Chronic treatment, on the other hand, increased the time spent in the white compartment at the higher dose (Fig. 1C, $F_{[2, 29]} = 15.92$, $p < 0.0001$) without producing effects on locomotion (Fig. 1D, $F_{[2, 29]} = 0.07237$, NS).

The smaller dose of chlordiazepoxide (0.02 mg kg^{-1}) significantly decreased white avoidance, but the highest dose did not (Fig. 2A, $F_{[2, 29]} = 8.01$, $p = 0.0019$). No locomotor effects were observed for any dose (Fig. 2B, $F_{[2, 29]} = 1.157$, NS). Clonazepam decreased white avoidance in the smallest dose (Fig. 2C, $F_{[3, 39]} = 5.596$, $p = 0.003$). The highest dose of clonazepam, on the other hand, seemed to produce ataxia or sedation, as it decreased the frequency of shuttle events (Fig. 2D, $F_{[3, 39]} = 4.367$, $p = 0.0101$). Diazepam decreased white avoidance at 1.25 mg kg^{-1}, but not 2.5 mg kg^{-1} (Fig. 2E, $F_{[2, 29]} = 6.241$, $p = 0.0059$); no locomotor effects were observed (Fig. 2F, $F_{[2, 29]} = 0.6199$, NS).

Buspirone produced an anxiolytic effect (that is, increased time spent in the white compartment) in both doses studied (Fig. 3A, $F_{[2, 29]} = 53.94$, $p < 0.0001$) without producing locomotor impairment (Fig. 3B, $F_{[2, 29]} = 0.4077$, NS). The MAO-A inhibitor moclobemide did not produce effects on either white avoidance (Fig. 3B and C, $F_{[2, 29]} = 1.234$, NS) nor locomotion (Fig. 3D, $F_{[2, 29]} = 0.1944$, NS).

Acute ethanol treatment increased the time spent in the white compartment at the smallest concentrations (0.25% and 0.5%), but did not have effects at 1.0% (Fig. 4A, $F_{[3, 39]} = 37.56$, $p < 0.0001$); there was a biphasic effect on locomotion, with increase at 0.5% and a non-significant decrease at 1.0% (Fig. 4B, $F_{[3, 39]} = 7.687$, $p = 0.0004$).

Caffeine produced an anxiogenic (Fig. 5A, $t_{[df = 18]} = 3.139$, $p = 0.0057$), but not a locomotor effect (Fig. 5B, $t_{[df = 16]} = 0.5226$, $p = 0.6084$).

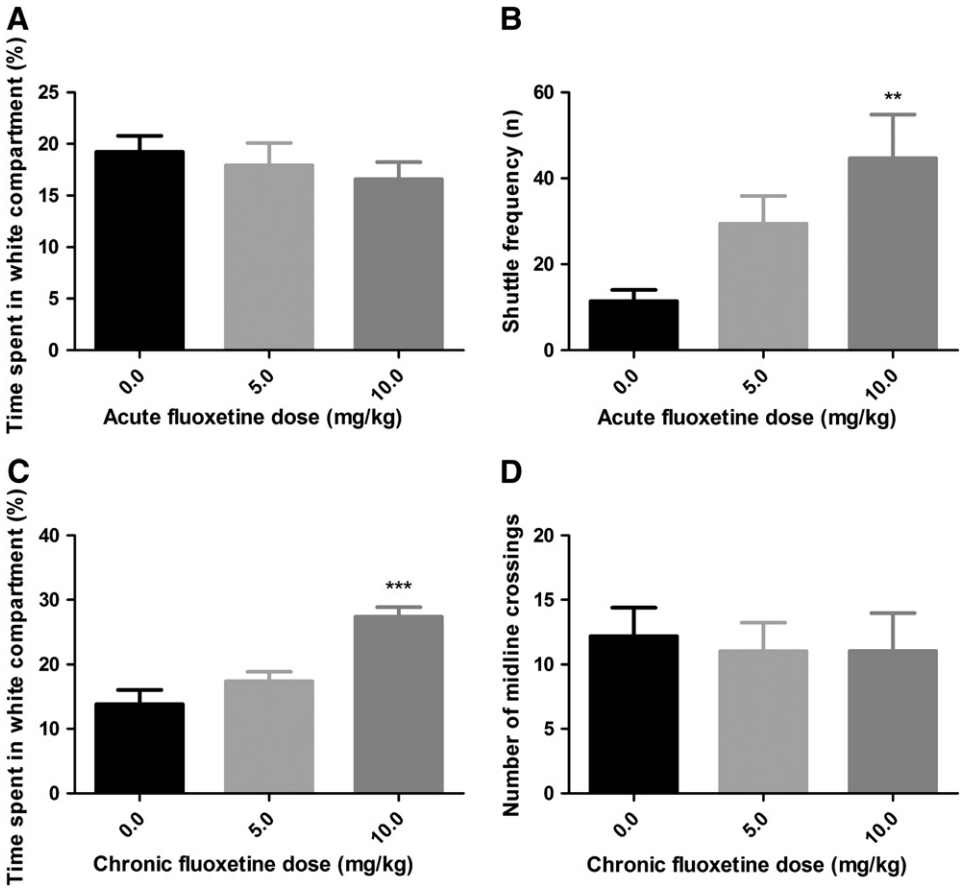

Fig. 1. Acute (A and B) and chronic (2 weeks; C and D) fluoxetine treatment ($n = 10$ each) produce a behavioral effect in zebrafish at the highest dose (10.0 mg kg^{-1}). Acute fluoxetine increased locomotion (B), while chronic treatment (2 weeks) increased the time spent in the white compartment (C). **$p < 0.01$, ***$p < 0.0001$, one-way ANOVA with Dunnet's post-hoc test.

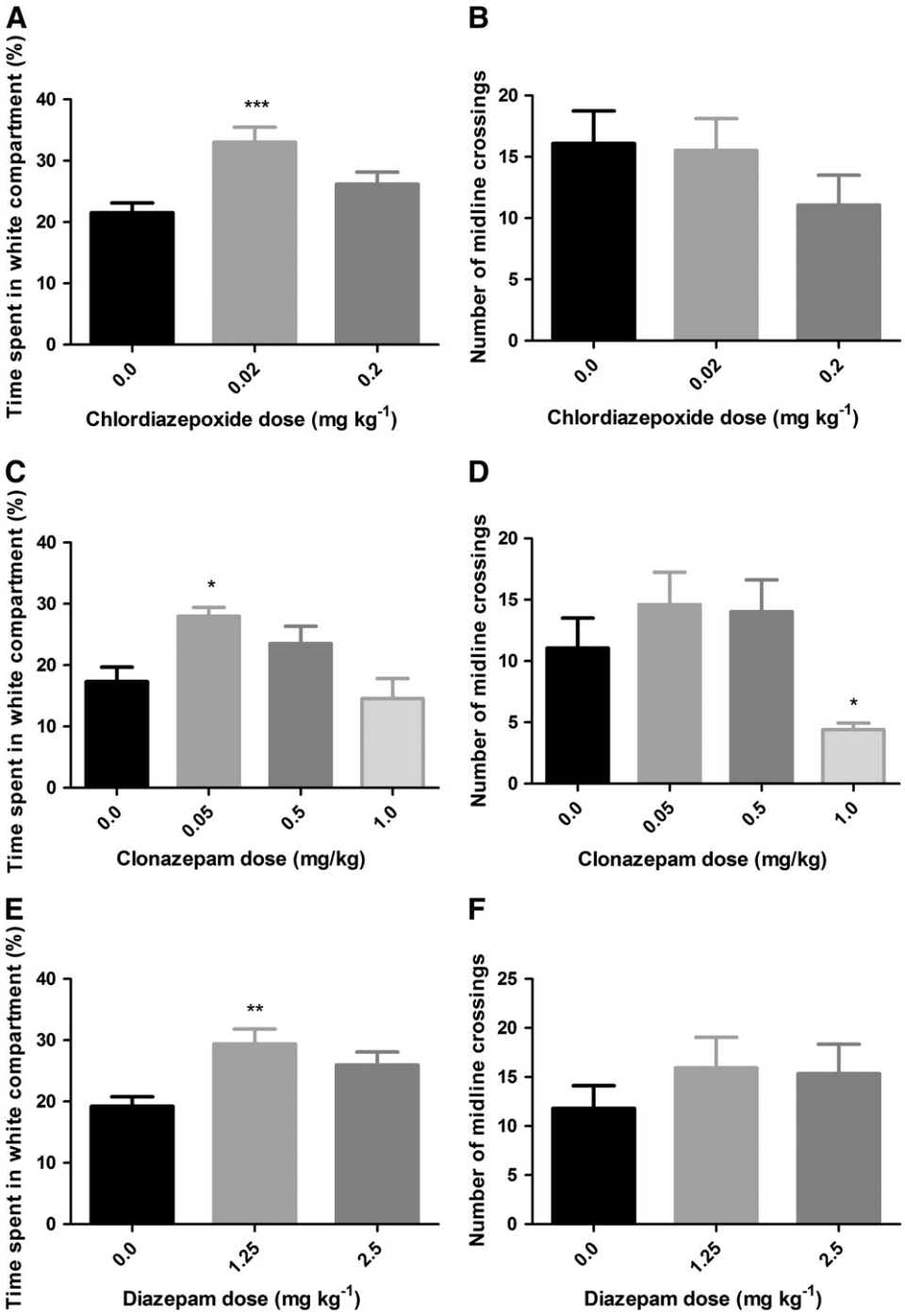

Fig. 2. Effect of benzodiazepines on white avoidance (A, C, and E) and locomotor activity (B, D, and F) in the scototaxis in zebrafish ($n = 10$ each). Chlordiazepoxide, clonazepam and diazepam produce a hormetic anxiolytic effect. Clonazepam produced a locomotor impairment at the highest dose (1.0 mg kg^{-1}) (D). *p<0.05, **p<0.01, ***p<0.0001, one-way ANOVA with Dunnet's post-hoc test.

4. Discussion

4.1. Serotonergic drugs in scototaxis

Fluoxetine, a selective serotonin reuptake inhibitor, produced a pattern of response that is similar to what is observed in mammals (including humans) — that is, an anxiolytic effect only in chronic treatment (Fig. 1). These results are consistent with what was observed in the novel tank diving test in zebrafish (Egan et al., 2009) and in the open-field in Chinook salmon (Clements and Schreck, 2007). The lack of acute effect is consistent with the effect of another SSRI, citalopram, which did not produce an anxiolytic effect in a modified version of the dark/light box with acute treatment (Sackerman et al., 2010). Likewise, acute fluoxetine has no effect in the novel tank diving test (Stewart et al., in press b), but citalopram does (Sackerman et al., 2010); this difference in profiles could be explained by differences in binding site (allosteric versus orthosteric) occupied by these drugs in the serotonin transporter. Acute fluoxetine, however, tends to increase anxiety in rodent models (Burghardt et al., 2007; Drapier et al., 2007; Silva and Brandão, 2000), as it does in an active avoidance task in goldfish (Beulig and Fowler, 2008).

A possible explanation for this disparity is that zebrafish has two serotonin transporters with complementary distributions, coded by

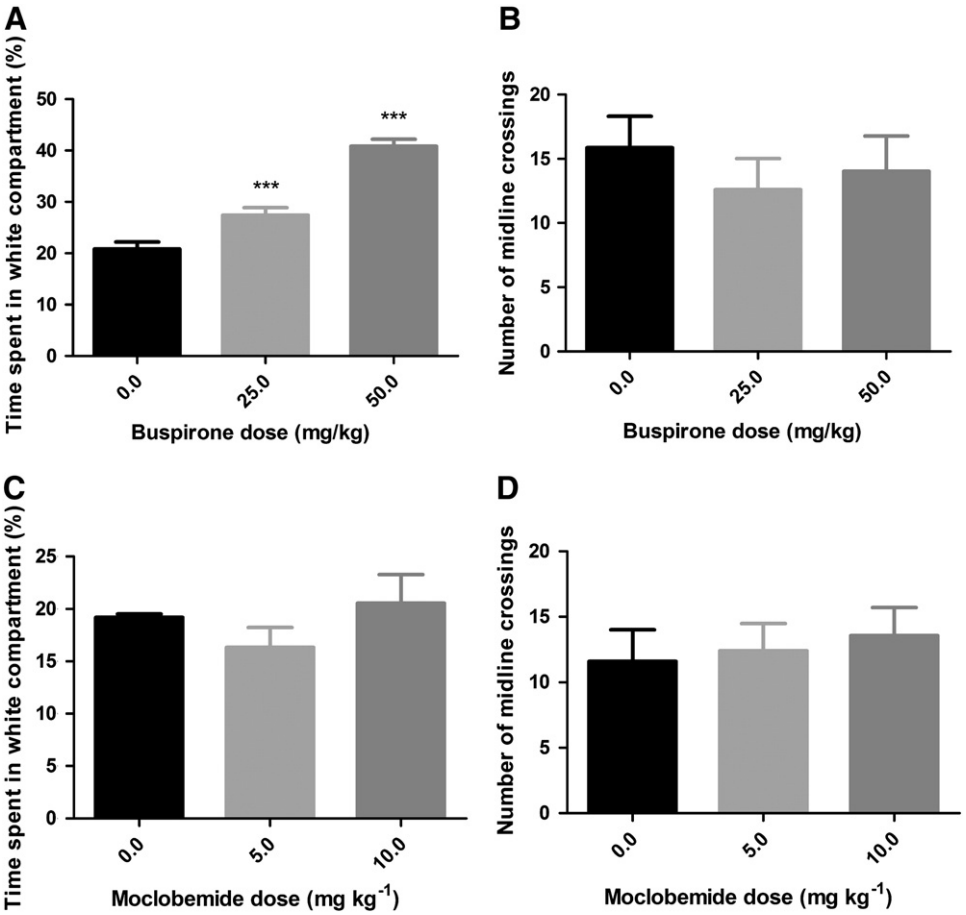

Fig. 3. Effect of buspirone (A and B) moclobemide (C and D) and on white avoidance and locomotor activity in the scototaxis in zebrafish ($n = 10$ each). Buspirone produced an anxiolytic effect without locomotor impairment, while moclobemide did not produce behavioral effects at the doses tested. ***$p < 0.0001$, one-way ANOVA with Dunnet's post-hoc test.

the genes *slc6a4a* and *slc6a4b* (Norton et al., 2008; Wang et al., 2006); the first is expressed in the superior and inferior raphe, and dorsal and ventral parts of the periventricular pretectal nucleus, while the second is expressed mainly in the paraventricular organ, retina and medulla oblongata (Norton et al., 2008; Wang et al., 2006). It seems, then, that zSERTA controls serotonin reuptake in the central nervous system, while zSERTB controls reuptake in the cerebrospinal fluid (Norton et al., 2008). Site-directed mutagenesis experiments (Severinsen et al., 2008) pointed that residues Ala^{505}, Leu^{506} and Ile^{507} in the zSERTA receptor are responsible for its three-fold increase in K_m and two-fold increase in V_{max} in relation to hSERT; these residues are absent in the zSERTB receptor, which probably has different affinities for 5-HT and SSRIs than its counterpart. These probable differences in affinity should explain the low potency of acute fluoxetine in inducing anxiety in zebrafish.

Interestingly, zSERTA also shows a higher affinity for imipramine (an reuptake blocker with greater affinity for the noradrenaline transporter than the serotonin transporter) and desipramine (an reuptake blocker with greater affinity for the serotonin transporter than the noradrenaline transporter) in relation to hSERT (Sackerman et al., 2010; Severinsen et al., 2008); consistent with that, in a modified version of the light/dark box, desipramine did not change the total time the animal spent in the white compartments or the number of entries in the white compartments (Sackerman et al., 2010); on the other hand, both acute desipramine and acute citalopram had anxiolytic effects in the novel tank diving test (Sackerman et al., 2010).

Buspirone also produced an anxiolytic effect on this test, without any effects on locomotion (Fig. 3A, B). The doses used (25 and 50 mg kg^{-1}) were chosen based on previous work on the effects of buspirone on the novel tank diving test (Bencan et al., 2009). Buspirone is thought to preferentially bind 5-HT_{1A} autoreceptors in the raphe, where it acts as a partial agonist to reduce the synthesis and release of serotonin (Meller et al., 1990). As is the case with the duplication of serotonin transporters, zebrafish also posses two different 5-HT_{1A} receptors, coded by the genes *htr1aa* and *htr1ab* (Norton et al., 2008). Both receptors are expressed in the superior raphe, which could explain why, despite of the duplication event, buspirone still produces anxiolysis in both scototaxis and the novel tank diving test.

Other serotonergic drugs which produce effects on zebrafish anxiety are LSD (a partial agonist at 5-HT_{2A} receptors) (Grossman et al., 2010), selegiline (a MAO-B inhibitor), clorgyline (a MAO-A inhibitor), fluvoxamine (a selective serotonin reuptake inhibitor) and *para*-chlorophenylalanine (PCPA, a tryptophan hydroxylase inhibitor) (Sallinen et al., 2009). In adult zebrafish, LSD produces an anxiolytic-like profile in the novel tank diving test, in the scototaxis test, and in the open-field, but it paradoxically increased whole-body cortisol levels (Grossman et al., 2010). Selegiline and clorgyline decreased locomotion and bottom-dwelling in larval zebrafish, while also decreasing monoamine oxidase activity, increasing heart rate and 5-HT levels (Sallinen et al., 2009), while co-treatment with PCPA rescues behavioral alterations and serotonin levels. In adult zebrafish, acute treatment with the non-selective, irreversible MAO inhibitor tranylcypromine produces anxiolysis at low doses and sedation at high doses (Stewart et al., in pressb). Selegiline and clorgyline act as monoamine oxidase inhibitors, and zebrafish possess only one copy of this enzyme (as opposed to the two isoforms observed in mammals) (Anichtchik et al., 2006; Setini et al., 2005). *In vitro* and *in vivo* pharmacological analyses, as well as sequence data, suggest that

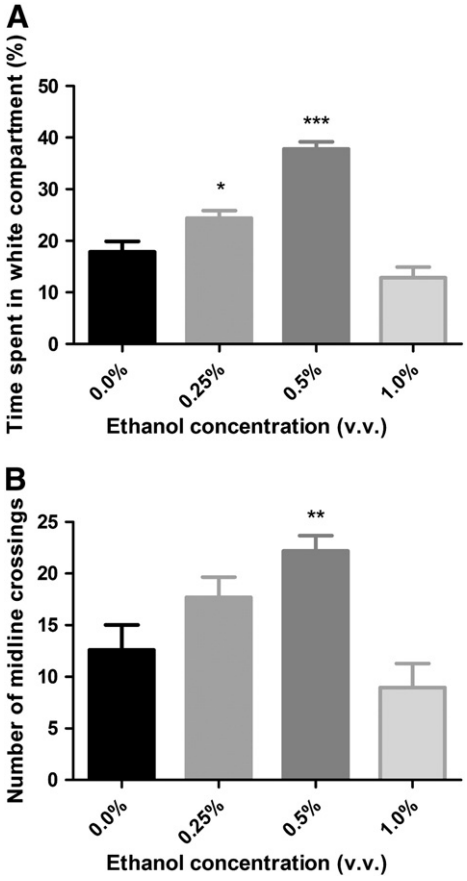

Fig. 4. Acute ethanol treatment produces an anxiolytic (at 0.25% and 0.5% v.v.) and hyperlocomotor (0.5% v.v.) effect on the scototaxis test ($n = 10$ each). *p<0.05, **p<0.01, ***p<0.0001, one-way ANOVA with Dunnet's post-hoc test.

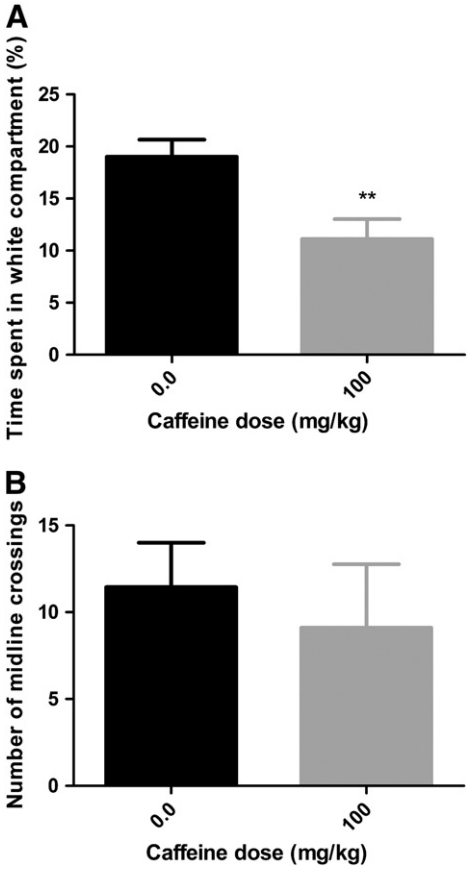

Fig. 5. Acute caffeine (100 mg kg^{-1}) is anxiogenic, but not locomotor activating, in the scototaxis test ($n = 10$ each). **p<0.01, one-way ANOVA with Dunnet's post-hoc test.

zMAO is more akin to human MAO-A than MAO-B (Anichtchik et al., 2006; Sallinen et al., 2009; Setini et al., 2005), which explains the selective effects of MAO inhibitors on serotonin levels. In the present article, however, MAO inhibition did not produce any effects on zebrafish behavior (Fig. 3C, D). These results are different from what is observed in the novel tank diving test (Sallinen et al., 2009; Stewart et al., in pressb), suggesting a difference in the types of anxiety which are modeled by each test. Indeed, monoamine oxidase inhibitors are thought to be effective against panic disorder, but not generalized anxiety disorder (Lieberman and Tasman, 2006).

4.2. Benzodiazepines and ethanol in scototaxis

Benzodiazepines produced an anxiolytic-like hormetic profile in the scototaxis test (Fig. 2), a predominant result with benzodiazepines (Calabrese, 2008). The highest dose of clonazepam produced a locomotor impairment, with the smallest dose (0.05 mg kg^{-1}) producing anxiolysis. At doses equivalent to those used in the present experiments, diazepam, but not chlordiazepoxide, was effective in the novel tank diving test (Egan et al., 2009; Sackerman et al., 2010). In a modified version of the scototaxis test, chlordiazepoxide increased the number of entries in the white compartments and the time spent there (Sackerman et al., 2010). Not surprisingly, pentylenetetrazole, a GABA antagonist, increases the time spent in the top and impairs habituation of bottom-dwelling and erratic movements in the novel tank diving test (Wong et al., 2010).

Among other effects, acute ethanol treatment affects $GABA_A$ and NMDA receptors (Krystal and Tabakoff, 2002). In this article, ethanol produced a biphasic effect on both anxiety and locomotion (Fig. 4). These results parallel the anxiolytic effect of 0.5% ethanol on the modified scototaxis test (Sackerman et al., 2010) and in the novel tank diving test (Egan et al., 2009), as well as the biphasic locomotor effect in an open-field (Gerlai et al., 2000). The locomotor-activating effect of ethanol, in zebrafish larvae, seems to be dependent on adenylate cyclase-mediated phosphorylation of extracellular signal-regulated kinases (ERKs), while the locomotor-depressing effects are not (Peng et al., 2009). Ethanol, however, presents effects on many different systems, including $GABA_A$-Rs, NMDA-Rs, μ-opioid receptors, dopamine and norepinephrine receptors, and voltage-sensitive calcium receptors (Krystal and Tabakoff, 2002). Allied to the fact that the anxiolytic-like effect is accompanied by a hyperlocomotor effect at the 0.5% concentration, this makes it difficult to interpret the outcomes of acute ethanol intoxication in zebrafish.

4.3. Caffeine in scototaxis

Caffeine is a non-selective antagonist at A_1, A_{2A} and A_{2B} adenosine receptors, also inhibiting phosphodiesterases (Daly et al., 1999). In zebrafish, caffeine produces an anxiogenic effect on the scototaxis test (Stewart et al., 2010) and in the novel tank diving test (Egan et al., 2009; Wong et al., 2010); an inverted U-shaped dose–response function was observed in locomotion in the scototaxis test (Stewart et al., 2010). The anxiogenic effects were attributed to antagonism at A_1 receptors, since DPCPX (a selective A_1-R antagonist) also increases anxiety; the locomotor effect was attributed to A_{2A} receptors, since ZM241,385 (an A_{2A}-R antagonist) increased locomotion, but not anxiety (Stewart et al., 2010). In the present work, these results were replicated, with the caffeine dose used (100 mg kg^{-1}) being effective in producing an anxiogenic, but not hyperlocomotor effect (Fig. 5).

5. Conclusion

This work presented the effects of drugs which are used in the clinical treatment of anxiety disorders, including benzodiazepines, fluoxetine and buspirone, as well as investigated the modulation of anxiety by ethanol and caffeine. Overall, the results obtained were consistent with results obtained in rodent models, such as the murine light–dark box (Bourin and Hascöet, 2003), and in the novel tank diving test (Bencan et al., 2009; Cachat et al., 2010; Egan et al., 2009; Stewart et al., in press b). Some differences in the pharmacology of the novel tank diving test and the scototaxis test are observed; the novel tank diving test, for example, seems to be sensitive to diazepam, but no chlordiazepoxide, while our results show that the scototaxis test is sensitive to other benzodiazepines as well. In the novel tank diving test, citalopram (a selective serotonin reuptake inhibitor) is anxiolytic in acute treatment, while fluoxetine did not show the same effect in the present work and in the novel tank test; our results are consistent with the time course of SSRIs in the clinical management of anxiety disorders. Our results also show a lack of sensitivity for moclobemide, a MAO-A inhibitor, which suggest selectivity for anxiolytic (and not panicolytic) drugs.

Overall, the present results show the feasibility of the scototaxis test for pharmacological assessment of anxiety. At the present moment, the scototaxis test represents a reliable and low-cost alternative for behavioral phenotyping in zebrafish. Also, the fact that drugs which have clinical efficacy on generalized anxiety disorder but not on panic disorder (e.g., buspirone, early benzodiazepines) or vice-versa (e.g., moclobemide), as well as the previous observations that the preference for dark environments is better explained by a approach–avoidance conflict and not simply a reaction to novelty (Blaser et al., 2010; Maximino et al., 2010b,c), suggest that the scototaxis test could be a model of generalized anxiety in zebrafish.

Acknowledgments

This work was supported by Conselho Nacional de Pesquisa, CNPq/Brazil (grants 483336/2009-2 and 400039/2009-5). CM is the recipient of a studentship grant from Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior, CAPES/Brazil. AGJ is the recipient of a productivity scholarship from Conselho Nacional de Pesquisa, CNPq/Brazil.

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