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Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety

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ABSTRACT

Zebrafish are becoming more widely used to study neurobehavioral pharmacology. We have developed a method to assess novel environment diving behavior of zebrafish as a model of stress response and anxiolytic drug effects. In a novel tank, zebrafish dwell in the bottom of the tank initially and then increase their swimming exploration to higher levels over time. We previously found that nicotine, which has anxiolytic effects in rodents and humans, significantly lessens the novel tank diving response in zebrafish. The specificity of the diving effect was validated with a novel vs. non-novel test tank. The novel tank diving response of zebrafish was tested when given three anxiolytic drugs from two different chemical and pharmacological classes: buspirone, chlordiazepoxide and diazepam. When the test tank was novel the diving response was clearly seen whereas it was significantly reduced when the test tank was not novel. Buspirone, a serotonergic ($5HT_{1A}$ receptor agonist) anxiolytic drug with some D_2 dopaminergic effect, had a pronounced anxiolytic-like effect in the zebrafish diving model at doses that did not have sedative effects. In contrast, chlordiazepoxide, a benzodiazepine anxiolytic drug, which is an effective agonist at GABA-A receptors, did not produce signs of anxiolysis in zebrafish over a broad dose range up to those that caused sedation. Diazepam another benzodiazepine anxiolytic drug did produce an anxiolytic effect at doses that did not cause sedation. The zebrafish novel tank diving task can be useful in discriminating anxiolytic drugs of several classes (serotonergic, benzodiazepines and nicotinic).

1. Introduction

Zebrafish (*Danio rerio*) are becoming widely used for the study of the molecular bases of neurobiology with applications in neuropharmacology and neurotoxicology (Linney et al., 2004; Teraoka et al., 2003). As a model organism, the zebrafish provides an inexpensive, potentially high-throughput test subject that could prove useful in gaining a greater understanding of neuropharmacological mechanisms in mammals and facilitate drug discovery. Zebrafish studies have recently begun use assessments of complex behavioral functions such as, learning, memory and anxiety response (Arthur and Levin, 2001; Bass and Gerlai, 2008; Bencan and Levin, 2008; Colwill et al., 2005; Dahm and Geisler, 2006; Eddins et al., 2009; Hicks et al., 2006; Levin and Chen, 2004; Levin et al., 2007; Levin et al., 2003; Levin et al., 2006; López-Patiño et al., 2008; Ninkovic and Bally-Cuif, 2006; Peitsaro et al., 2003; Pradel et al., 1999; Pradel et al., 2000; Rawashdeh et al., 2007; Williams et al., 2002; Xu et al., 2007). Development of validated behavioral tests for complex behavioral function in zebrafish will facilitate the analysis of the neural and molecular mechanisms of behavioral function as well as economical screening for promising new drugs for treating behavioral dysfunction.

Anxiety and stress responses are beginning to be investigated in zebrafish. Anxiety-like behavior in zebrafish has been shown through patterns of swimming along the edge (Pietsaro et al., 2003) and towards the bottom of novel environments (Levin et al., 2007). Nicotine at a dose of 50 mg/l given by immersion for 3 min caused a significant attenuation of the diving response (time spent dwelling in the bottom of the tank) during the initial part of the 5-minute test session. The higher 100 mg/l nicotine caused a significant reduction in the bottom dwelling throughout the session. There is typically increased swimming activity over the course of the test session. This was significantly attenuated by both 50 and 100 mg/l of nicotine. These types of behaviors are not seen as frequently when the fish had previously been exposed to its surroundings. We are using this paradigm in zebrafish to determine the efficacy of different classes of anxiolytics. Nicotine has been shown in our previous study to reduce anxiety in zebrafish placed in a novel environment and could be reversed by the nicotinic acetylcholine receptor antagonist, mecamylamine (Levin et al., 2007). The 100 mg/l nicotine dose has previously been seen in our studies to significantly improve learning and memory in zebrafish (Levin et al., 2007; Levin et al., 2006).

Zebrafish can be used to study the neural mechanisms of a variety of types of behavior. Key to this effort is the development of sensitive, efficient and reliable tests of the behavioral functions of interest. We have found that the novel tank diving response of zebrafish can

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provide insight into stress reactivity and anxiety (Levin et al., 2007). The diving response to dwell in the bottom of a novel tank and subsequently explore higher levels of the tank resembles thigmotaxis (position choice along the wall) seen in rodents upon initial presentation into a novel open field that is commonly interpreted as anxiety response to stress (Simon et al., 1994; Treit and Fundytus, 1988). Previously, we found that nicotine administration at a dose level, which significantly improves learning and memory (Levin and Chen, 2004; Levin et al., 2006) significantly attenuates this diving response (Levin and Chen, 2004). This is congruent with the findings of anxiolytic effects of nicotine in rodents and humans. To determine the similarities and differences in the neural bases of anxiety and stress response in zebrafish with mammals we examined broad dose effects of three different drugs with anxiolytic effects in humans and rat models: chlordiazepoxide, diazepam and buspirone.

Benzodiazapine receptors have been found in a wide variety of species including boney fish but not lower vertebrates and invertebrates (Nielsen et al., 1978). A variety of studies have identified benzodiazepine-GABA-A receptors in fish and shown that they have similar binding characteristics as those in rodents and humans (Anzelius et al., 1995a; Anzelius et al., 1995b; Carr et al., 1999; Friedl et al., 1988; Wilkinson et al., 1983), though functionally they may have some differences from mammals (Betti et al., 2001). There is evidence that benzodiazepine-GABA receptors are less pharmacologically responsive in fish, at least with regard to controlling convulsant activity (Corda et al., 1989). With regard to potential anxiolytic effects, Rehnberg et al. found in fathead minnows that exposure to the fish alarm pheromone reduced swimming activity in control fish but that the reduction in swimming effect was not seen in fish after exposure to 20 mg/l of chlordiazepoxide for an hour (Rehnberg et al., 1989). However, in their experiment, that dose of chlordiazepoxide had decreased activity relative to controls before the alarm pheromone was given.

The current study was conducted to test the effect of two different types of anxiolytic drugs on the novel environment diving response in zebrafish to determine the involvement of benzodiazepine and serotonergic systems in this zebrafish model of anxiety. Specifically, we studied extensive dose-effect functions with chlordiazepoxide, and diazepam a benzodiazepine anxiolytics, and buspirone, a serotonergic anxiolytic, on the novel environment diving response as well as swimming activity. This study together with our previous work with nicotine will help determine the applicability of this zebrafish model across anxiolytic drug classes and the participation of different transmitter receptor systems in anxiolytic response in zebrafish.

2. Methods

2.1. Subjects

Zebrafish (*D. rerio*) obtained from Triangle Tropical Fish (Durham, NC) were kept at approximately 28.5 °C on a 12:12-h light/dark cycle in an automated flow-through continuously filtered water system by Aquatic Habitats (Apopka, FL, USA). Behavioral testing of drug effects took place during the light phase between 8:00 a.m. and 5:00 p.m. In each study the fish were randomly sorted into treatment groups and vehicle-treated controls so that there was no confounding of breeding or holding conditions with drug treatment. The tank water was made by mixing de-ionized H_2O and sea salts (Instant Ocean, 1.2 g/20 l of H_2O). The tanks with the groups of adult fish were maintained with constant filtration and aeration. The size of the housing tanks for the fish were 1.5 l and 3 l in Experiment 1 so that the effect of home tank size on the novel tank diving behavioral test could be compared. The tanks used in the diving test was always the same-sized 1.5-liter tanks. In Experiments 2 and 3 in which we tested drug dose effect of diving, all of the fish were housed in 3-liter tanks. Fish were fed twice daily with brine shrimp and flake fish food. Again the tank used in the diving test was always the same-sized 1.5-liter tanks.

2.2. Experimental overview

Experiment 1 was a study of the impact of home tank housing tank size on the dynamics of diving response during the novel tank diving test. The critical measure for the diving response was the proportion of time the fish spent dwelling in the bottom third of the test tank. Experiment 2 characterized the effects of a broad dose-effect function of the serotonergic anxiolytic buspirone on diving response (time spent dwelling in the bottom third of the tank) and swimming activity in the novel tank diving test. Experiment 3 characterized the effects of a dose-effect function of benzodiazepine drug chlordiazepoxide on diving response and swimming activity in the novel tank diving test. Experiment 4 characterized the effects of a broad dose-effect function of the widely-used benzodiazepine drug diazepam on diving response and swimming activity in the novel tank diving test.

2.3. Test apparatus and procedure

The zebrafish were placed in one of two 1.5-liter plastic tanks filled with 1350 ml of home tank water from the fish housing apparatus. Each tank was a trapezoid: 22.9 cm along the bottom, 27.9 cm at the top, 15.2 cm high and 15.9 cm along the diagonal side. It was 6.4 cm wide at the top, and tapered to 5.1 cm at the bottom. The tanks were positioned so that the diagonal sides were facing each other, with a sheet of white paper obstructing the view into the other tank. The tanks were backlit and had a translucent white sheet of plastic serve as a background for the imaging system. They were located 88.5 cm from the Samsung 8 mm Camcorder used to record the image into the Noldus Image Analysis program, EthoVision (Wageningen, The Netherlands). Swimming behavior (tank location choice and locomotor speed) was assessed by the Noldus software. Time per minute of the test spent in each third of the tank sectioned horizontally as well as swim path length per minute (log cm/min) were the dependent measures. The log of activity was taken to normalize the data. Since activity data are typically skewed it is appropriate that they are analyzed as the log of the raw score. The choice of position (bottom vs. upper levels, see Fig. 1) was considered the index of anxiety. Choice of dwelling on the bottom was near a position of safety similar to the position choice of closed vs. open arms in the elevated plus maze and positions near the wall (thigmotaxis) vs. the center of an open field with rodents. As with the elevated plus maze and open field in rodents there was a separate total activity measure as well.

2.4. Drug administration

Buspirone HCl (Sigma, St. Louis, MO, USA) was administered by immersing the zebrafish in a beaker with concentrations of 0, 3.125,

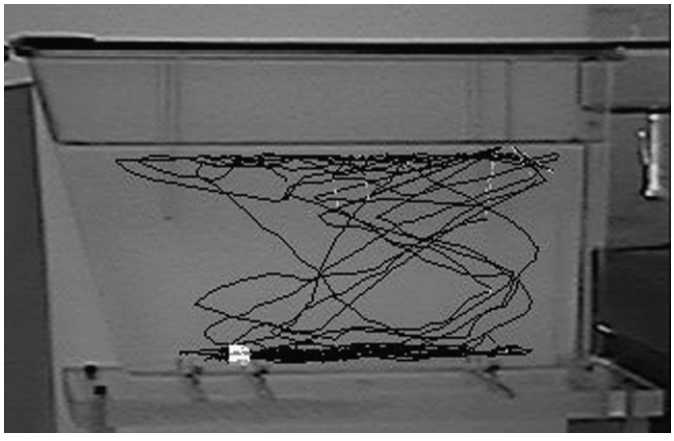

Fig. 1. Computerized video track of a zebrafish's path in the novel tank over 5 min.

6.25, 12.5, 25 and 50 mg/l for 3 min. Chlordiazepoxide HCl (Sigma, St. Louis, MO, USA) was administered in the same fashion with concentrations of 0, 0.625, 1.25, 2.5, 5, 10 and 20 mg/l for 3 min. The dose of the drug was calculated from the weight of the salt. Diazepam HCl (Sigma, St. Louis, MO, USA) was administered in the same fashion with concentrations of 0, 0.625, 1.5, 2.5, 5, 10 and 20 mg/l for 3 min. The dose of the drug was calculated from the weight of the salt. A delay of 5 min was imposed between the end of dosing and the start of the trial. Tank water was used as the vehicle for buspirone and chlordiazepoxide and tank water with 5% DMSO was used as the vehicle for diazepam to facilitate solution of the drug. The fish were exposed to the drug in a separate beaker and then were put into a holding tank without drug for the interval between exposure and testing. Exposure to tank water without drug added served as the control. There was no drug exposure in either the home tank or the test chamber. All the fish were drug naïve and each fish was used only once. There were at least 10 fish per condition.

2.5. Data analysis

The data were analyzed by a mixed design analysis of variance with between subject factors of drug, drug dose and timing of drug administration. The repeated measure was minute within the 5-minute session. The two dependent measures were seconds per minute that the fish spent at the bottom of the test tank and swim speed (cm/min). A cut-off of $p<0.05$ (two-tailed) was used as the threshold for statistical significance. Linear trend analysis was used to determine the time and dose effects. Dunnett's test (two-tailed) was used to compare the effects of drug doses vs. control.

3. Results

3.1. Experiment 1: importance of novelty of the test tank

To document the importance of novelty of the test tank we conducted a study testing zebrafish, which were housed in the same-sized 1.5-liter tanks in which they were tested ($N=50$). Thus, the tanks were not novel in the sense of size and dimensions. Their responses to being placed in the test tank were compared with fish, which were housed in larger 3-liter tanks, which were twice as wide as the 1.5-liter tanks ($N=20$). All fish were tested in identical 1.5-liter, narrow tanks. All the fish had the same procedure of netting them from their home tank and placing them in the test tank. As shown in Fig. 2, there was a significant ($F(4,272)=7.27$, $p<0.0001$) interaction of home tank size × minute of the test with initial diving response followed by gradual increase in swimming to the upper

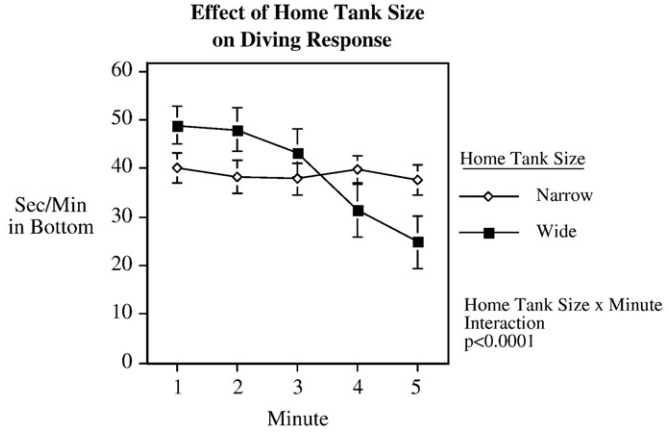

Fig. 2. Wide vs. narrow housing tank size effect on the diving response (s/min) in zebrafish (mean±sem).

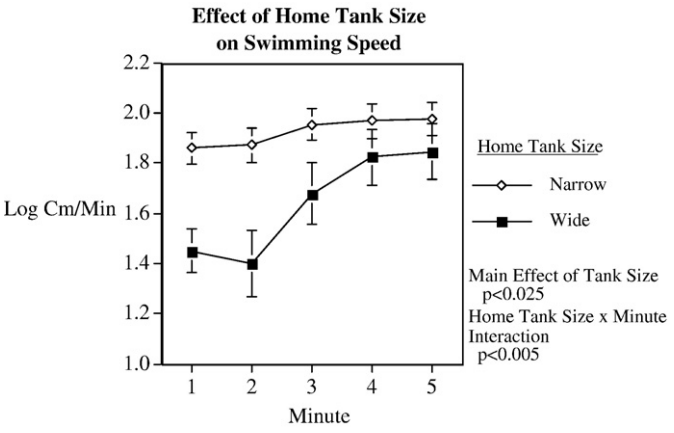

Fig. 3. Wide vs. narrow housing tank size effect on swimming activity (log cm/min) in zebrafish (mean±sem).

levels of the test tank in the fish housed in the larger tanks but not the smaller tanks. There was a significantly ($F(1,68)=14.30$, $p<0.0005$) greater linear trend of more bottom dwelling at the beginning of the session followed by less bottom dwelling as the session progressed in the fish with the wide home tanks opposed to those with narrow home tanks. Swimming speed was also differentially affected by the size of the home tank. As shown in Fig. 3, The fish from the narrow home tanks had significantly ($F(1,68)=6.88$, $p<0.025$) greater overall swim speeds in the 5-minute session than those from the wide home tanks. There was also a significant home tank size × minute of test interaction ($F(4,272)=4.60$, $p<0.005$) with the fish from the wide home tanks showing a greater decrease in speed during the early part of the session than later in the session. The linear trend of increasing speed across the successive minutes of the session in the fish from the wide home tanks was significantly ($F(1,68)=7.93$, $p<0.01$) greater compared to the fish from the narrow home tanks.

3.2. Buspirone

Buspirone caused a significant ($F(5,54)=6.47$, $p<0.001$) decrease in the diving response in a dose range that did not significantly affect swimming speed ($N=10$/dose). There was a significant ($F(1,54)=27.74$, $p<0.0001$) linear dose effect with higher buspirone doses causing a greater effect reducing bottom dwelling (Fig. 4). Individually compared with control with Dunnett's test buspirone doses of 6.25 mg/l ($p<0.05$), 25 mg/l ($p<0.01$) and 50 mg/l ($p<0.01$) caused significant decreases in diving compared to controls. Bottom dwelling in the group given 12.5 mg/l buspirone was somewhat lower than controls but not significantly so. This was likely due to random

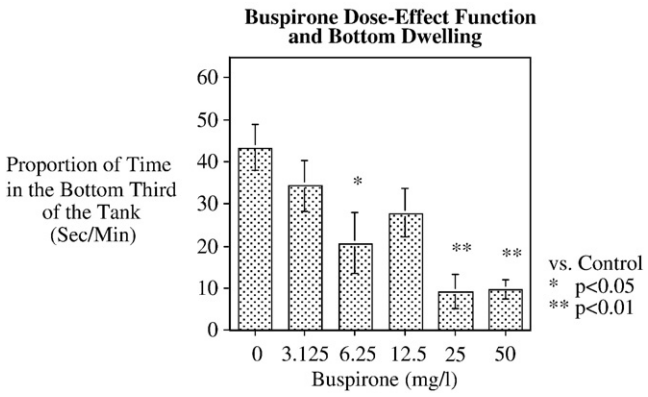

Fig. 4. Buspirone effects on bottom dwelling (s/min) in zebrafish (mean±sem).

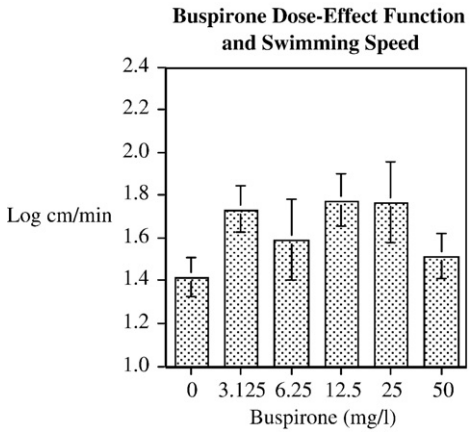

Fig. 5. Buspirone effects on swimming activity (log cm/min) in zebrafish (mean±sem).

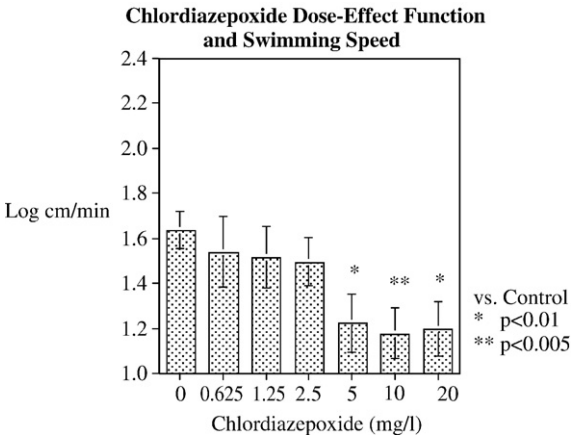

Fig. 7. Chlordiazepoxide effects on swimming activity (log cm/min) in zebrafish (mean±sem).

variability in response. In this dose range buspirone did not cause significant effects on swimming speed (Fig. 5).

3.3. Chlordiazepoxide

In a double dissociation from buspirone, which caused significant decreases in the diving response without significant sedation, chlordiazepoxide caused significant sedation but no significant effect on the diving response. Chlordiazepoxide stood in contrast to the effect of both buspirone in the current study and nicotine in the previous study that did not produce any change in the bottom dwelling over a wide dose range (Fig. 6). High doses of chlordiazepoxide caused a significant ($F(6,83)=2.90$, $p<0.025$) slowing in swimming by the zebrafish ($N=30$ controls and $N=10$ for each chlordiazepoxide dose). The linear trend of increased sedation with higher chlordiazepoxide doses was significant ($F(1,63)=12.48$, $p<0.001$). Significant sedation was caused by 5 mg/l ($p<0.01$), 10 mg/l ($p<0.005$) and 20 mg/l ($p<0.05$) of chlordiazepoxide (Fig. 7).

3.4. Diazepam

There was a significant reduction in bottom dwelling with diazepam ($F(6,93)=2.53$, $p<0.025$) with N's = 12–17/treatment group. The diazepam dose-effect function was biphasic with the relatively low to moderate doses of 1.25 ($p<0.025$) and 5 mg/l ($p<0.05$) causing a significant reductions in bottom dwelling relative to vehicle-treated controls (Fig. 8). This effect was not seen with lower or higher doses. Curiously the intermediate 2.5 mg/l diazepam dose did not cause a significant decrease in the time spent in the bottom of the test tank. This seemed likely due to random variability in the test groups. No significant effect of this dose range of diazepam was seen on swimming speed (Fig. 9).

4. Discussion

The current study demonstrated in a zebrafish model of stress response anxiolytic effects of two drugs: buspirone and diazepam which have been shown to be effective anxiolytics in humans and rodent models. A third drug, chlordiazepoxide was not found to be effective in this zebrafish model.

Chlordiazepoxide, a classic benzodiazepine anxiolytic, which acts as an effective agonist at GABA-A receptors did not show any signs of anxiolytic effects in the current zebrafish study over a wide dose range, although such effects are normally seen in rats (Treit and Fundytus, 1988). This lack of anxiolytic effect of chlordiazepoxide in zebrafish was not merely due to lack of penetrance into tissues. Chlordiazepoxide did have a significant behavioral effect in zebrafish. The full spectrum of sedative effect from no action to pronounced sedation was seen with this dose range given.

The lack of anxiolytic effect of chlordiazepoxide in this zebrafish model of anxiety response did not generalize to the class of benzodiazepine anxiolytics. Diazepam another member of this class did cause significant reduction in bottom dwelling. The dose-effect function of diazepam was U-shaped with low to moderate but not higher doses causing significant reductions in bottom dwelling. The anxiolytic effect of diazepam was seen in a dose range that did not affect swimming speed. Recently, diazepam has been found to have anxiolytic effects in another species of fish, goldfish (Faganello and Mattioli, 2007). In that study, lesions of the telencephalon did not

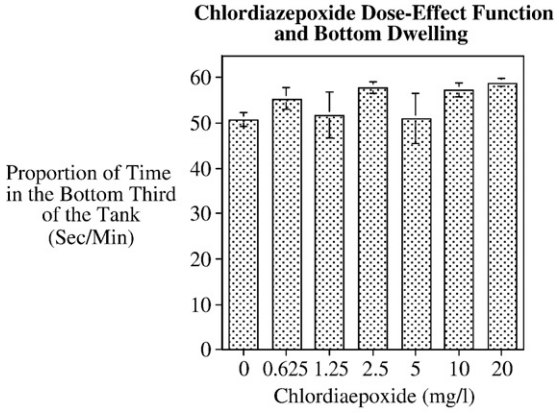

Fig. 6. Chlordiazepoxide effects on bottom dwelling (s/min) in zebrafish (mean±sem).

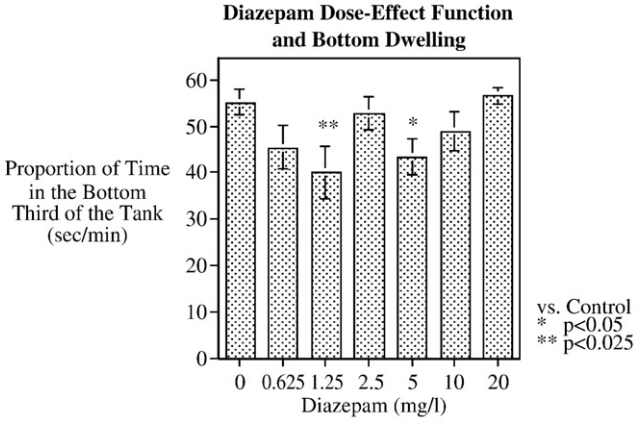

Fig. 8. Diazepam effects on bottom dwelling (s/min) in zebrafish (mean±sem).

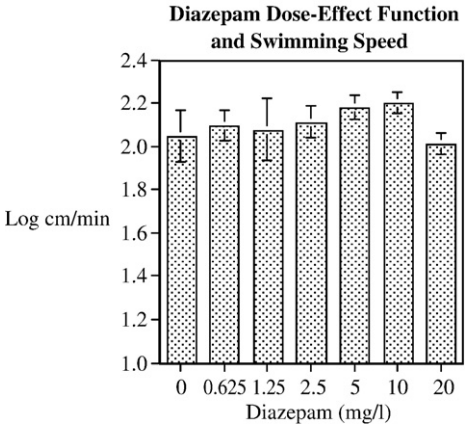

Fig. 9. Diazepam effects on swimming activity (log cm/min) in zebrafish (mean±sem).

alter the anxiolytic effect suggesting a more caudal locus of the diazepam effect, possibly in the diencephalon.

Interestingly, the non-benzodiazepine anxiolytic buspirone, which has actions mediated via serotonergic systems ($5HT_{1A}$ receptor agonist) as well as possible dopamine D_2 actions did show significant anxiolytic effects in the novel tank diving task. The dose-effect function was linear with greater effect with higher doses up to 50 mg/l given by immersion for 3 min. In a double dissociation relative to the effects of chlordiazepoxide, buspirone did not produce any significant effects on locomotor activity over this dose range.

In a methodological study, we found that home tank size makes a critical impact on the novel tank diving response. Fish housed in a narrow tank and tested in another narrow tank did not show the diving response or changes in swimming speed over the course of the five-minute session. This seems reasonable since their home tank and the test tank were identical. In contrast, fish housed in larger tanks twice the width of the narrow test tanks showed the typical novel tank diving response and recovery over the five-minute session. They also showed the typical slow swimming speed at the beginning of the test session followed by gradually increasing speed. This demonstrates a critical variable in reproducible use of the test. Testing in a novel environment appears to be essential for the test. The transfer procedure of netting the fish from their home tank and placing them into the test tank was the same for both groups. Thus, differential stress of transfer was unlikely to underlie the behavioral responses seen.

In another model of anxiety in zebrafish, increased anxiety response has also been seen in zebrafish in reaction to withdrawal from cocaine intoxication (López-Patiño et al., 2008). Interestingly, these investigators found that the anxiogenic effect of cocaine withdrawal was attenuated by diazepam, a benzodiazepine anxiolytic. Rehnberg et al. found in fathead minnows that 20 mg/l of chlordiazepoxide for an hour decreased the freezing response in reaction to exposure to the fish alarm pheromone, but this was relative to a lower baseline activity level before the alarm pheromone was administered (Rehnberg et al., 1989). In the current study we found that, chlordiazepoxide, was ineffective in attenuating anxiety response in the novel tank diving test. We did not see any sign of anxiolytic effect over a broad dose range covering the complete spectrum from no effect to a pronounced sedative effect. Like the Rehnberg study we found a sedative effect of 20 mg/l of chlordiazepoxide. With regard to the López-Patiño study, it may be the case that zebrafish are responsive to anxiolytic effects of some benzodiazepines but not others, there was a critical difference in the ways in which anxiety responding was assessed in the two studies. Increased distance traveled was the principal effect of cocaine withdrawal. This was counteracted by diazepam, which brought activity levels in the cocaine withdrawal down to below control levels. This is similar to the sedative effect we saw in the current study with another benzodiazepine drug chlordiazepoxide. In the current study we also found an anxiolytic effect of diazepam. It is important that there be studies with a variety of models of anxiety and drug treatments to better understand the neural bases of anxiety in zebrafish.

Future studies should explore the effects of other benzodiazepines for anxiolytic effects to help determine the critical differences between chlorpiazepoxide and diazepam. This test can also be applied to other drug candidates. Important to the development of the zebrafish model of stress response and anxiety as well as the use of this model to determine the neural mechanisms underlying stress response, anxiety and their treatment are further investigated of the neurochemical and neuromolecular underpinnings of these behavioral effects. The zebrafish can be particularly useful in these mechanistic studies, now that functional tests have been developed. The current and previous results suggest that anxiolytic effects of serotonergic and nicotinic anxiolytic drugs can be successfully detected. This study provides evidence for an inexpensive and high-throughput anxiety test with zebrafish, which can be used for identification of novel drug treatments for anxiety.

References

Anzelius M, Ekstrom P, Mohler H, Richards JG. Immunocytochemical localization of GABAA receptor beta 2/beta 3-subunits in the brain of Atlantic salmon (*Salmo salar* L). J Chem Neuroanat 1995a;8:207–21.

Anzelius M, Ekstrom P, Mohler H, Richards JG. Immunocytochemical localization of the GABAA/benzodiazepine receptor beta2/beta3 subunits in the optic tectum of the salmon. J Recept Signal Transduct Res 1995b;15:413–25.

Arthur D, Levin ED. Spatial and non-spatial discrimination learning in zebrafish (*Danio rerio*). Anim Cogn 2001;4:125–31.

Bass SL, Gerlai R. Zebrafish (*Danio rerio*) responds differentially to stimulus fish: the effects of sympatric and allopatric predators and harmless fish. Behav Brain Res 2008;186:107–17.

Bencan Z, Levin ED. The role of α7 and α4β2 nicotinic receptors in the nicotine-induced anxiolytic effect in zebrafish. Physiol Behav 2008;95:408–12.

Betti L, Giannaccini G, Gori M, Bistocchi M, Lucacchini A. [3H]Ro 15-1788 binding sites to brain membrane of the saltwater *Mugil cephalus*. Comp Biochem Physiol, Toxicol Pharmacol: Cbp 2001;128:291–7.

Carr RL, Couch TA, Liu J, Coats JR, Chambers JE. The interaction of chlorinated alicyclic insecticides with brain GABA(A) receptors in channel catfish (*Ictalurus punctatus*). J Toxicol Environ Health Part A 1999;56:543–53.

Colwill RM, Raymond MP, Ferreira L, Escudero H. Visual discrimination learning in zebrafish (*Danio rerio*). Behav Processes 2005;70:19–31.

Corda MG, Longoni B, Cau A, Paci S, Salvadori S, Laudani U, et al. Distribution and pharmacological properties of the GABAA/benzodiazepine/chloride ionophore receptor complex in the brain of the fish *Anguilla anguilla*. J Neurochem 1989;52:1025–34.

Dahm R, Geisler R. Learning from small fry: the zebrafish as a genetic model organism for aquaculture fish species. Mar Biotechnol 2006;8:329–45.

Eddins D, Williams P, Cerutti DT, Levin ED. Nicotine effects on learning in zebrafish: the role of dopamine systems. Psychopharmacology 2009;202:103–9.

Faganello FR, Mattioli R. Anxiolytic-like effect of chlorpheniramine in inhibitory avoidance in goldfish submitted to telencephalic ablation. Prog Neuro-Psychopharmacol Biol Psychiatry 2007;31:269–74.

Friedl W, Hebebrand J, Rabe S, Propping P. Phylogenetic conservation of the benzodiazepine binding sites: pharmacological evidence. Neuropharmacology 1988;27:163–70.

Hicks C, Sorocco D, Levin M. Automated analysis of behavior: a computer-controlled system for drug screening and the investigation of learning. J Neurobiol 2006;66:977–90.

Levin ED, Chen E. Nicotinic involvement in memory function in zebrafish. Neurotoxicol Teratol 2004;26:731–5.

Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. Physiol Behav 2007;90:54–8.

Levin ED, Chrysanthis E, Yacisin K, Linney E. Chlorpyrifos exposure of developing zebrafish: effects on survival and long-term effects on response latency and spatial discrimination. Neurotoxicol Teratol 2003;25:51–7.

Levin ED, Limpuangthip J, Rachakonda T, Peterson M. Timing of nicotine effects on learning in zebrafish. Psychopharmacology 2006;184:547–52.

Linney E, Upchurch L, Donerly S. Zebrafish as a neurotoxicological model. Neurotoxicol Teratol 2004;26:709–18.

López-Patiño MA, Yu L, Cabral H, Zhdanova IV. Anxiogenic effects of cocaine withdrawal in zebrafish. Physiol Behav 2008;93:160–71.

Nielsen M, Braestrup C, Squires RF. Evidence for a late evolutionary appearance of brain-specific benzodiazepine receptors: an investigation of 18 vertebrate and 5 invertebrate species. Brain Res 1978;141:342–6.

Ninkovic J, Bally-Cuif L. The zebrafish as a model system for assessing the reinforcing properties of drugs of abuse. Methods (Duluth) 2006;39:262–74.

Pietsaro N, Kaslin J, Anichtchik OV, Panula P. Modulation of the histaminergic system and behaviour by alpha-fluoromethylhistidine in zebrafish. J Neurochem 2003;86: 432–41.
Pradel G, Schachner M, Schmidt R. Inhibition of memory consolidation by antibodies against cell adhesion molecules after active avoidance conditioning in zebrafish. J Neurobiol 1999;39:197–206.
Pradel G, Schmidt R, Schachner M. Involvement of L1.1 in memory consolidation after active avoidance conditioning in zebrafish. J Neurobiol 2000;43:389–403.
Rawashdeh O, de Borsetti NH, Roman G, Cahill GM. Melatonin suppresses nighttime memory formation in zebrafish. Science 2007;318:1144–6.
Rehnberg BG, Bates EH, Smith RJ, Sloley BD, Richardson JS. Brain benzodiazepine receptors in fathead minnows and the behavioral response to alarm pheromone. Pharmacology Biochem Behavior 1989;33:435–42.
Simon P, Dupuis R, Costentin J. Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. Behav Brain Res 1994;61:59–64.
Teraoka H, Dong W, Hiraga T. Zebrafish as a novel experimental model for developmental toxicology. Congenital Anomalies 2003;43:123–32.
Treit D, Fundytus M. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacology Biochem Behavior 1988;31:959–62.
Wilkinson M, Wilkinson DA, Khan I, Crim LW. Benzodiazepine receptors in fish brain: [3H]-flunitrazepam binding and modulatory effects of GABA in rainbow trout. Brain Res Bull 1983;10:301–3.
Williams FE, White D, Messer WS. A simple spatial alternation task for assessing memory function in zebrafish. Behav Processes 2002;58:125–32.
Xu X, Scott-Scheiern T, Kempker L, Simons K. Active avoidance conditioning in zebrafish (*Danio rerio*). Neurobiol Learn Mem 2007;87:72–7.