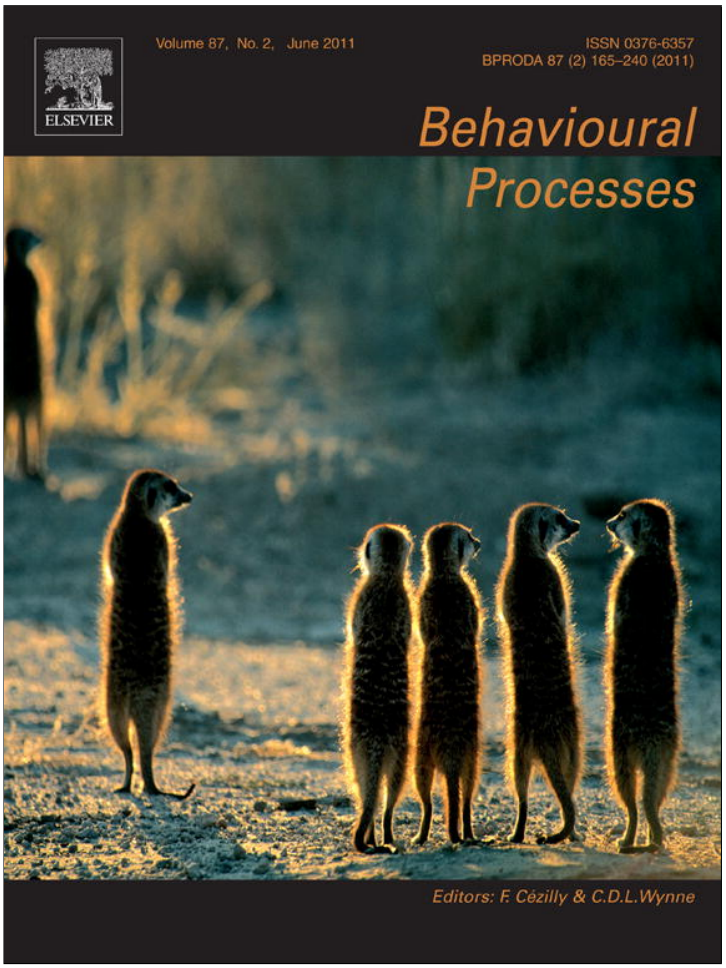

Behavioural Processes 87 (2011) 224–230

Contents lists available at ScienceDirect

Behavioural Processes

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

Acute stress disrupts performance of zebrafish in the cued and spatial memory tests: The utility of fish models to study stress–memory interplay

Siddharth Gaikwad, Adam Stewart, Peter Hart, Keith Wong, Valerie Piet, Jonathan Cachat, Allan V. Kalueff*

Department of Pharmacology and Neuroscience Program, Tulane Neurophenotyping Platform, Zebrafish Neuroscience Research Consortium (ZNRC), Tulane University Medical School, 1430 Tulane Avenue, New Orleans, LA 70112, USA

ARTICLE INFO

Article history:
Received 12 October 2010
Received in revised form 31 March 2011
Accepted 16 April 2011

Keywords:
Adult zebrafish
Spatial memory
Cued memory
Stress exposure
Alarm pheromone
Indian leaf fish
Plus-maze test

ABSTRACT

The zebrafish (*Danio rerio*) has emerged as a promising model organism for affective or cognitive neuroscience research, and may be useful to study the interplay between memory and anxiety-related states. To assess the effects of acute psychological stress on spatial and cued memory, adult zebrafish were trained in an aquatic plus-maze for 14 days using food bait as a reward. Two ecologically relevant stressors (alarm pheromone or Indian leaf fish exposure) were applied to acutely stress zebrafish immediately prior to the final (testing) trial. Overall, acute single inescapable stress markedly impaired spatial and cued memory in zebrafish plus-maze test, reducing the number of correct arm entries and time spent in the target arm. This observation parallels rodent and clinical literature on memory-impairing effects of acute stress, strongly supporting the utility of zebrafish in neurobehavioral research.

1. Introduction

Zebrafish (*Danio rerio*) are emerging as a new model species in experimental neuroscience (Egan et al., 2009; Gerlai et al., 2006; Piato et al., 2010). Representing the third most completely genetically characterized animal species after the mouse and the fruitfly (Grunwald and Eisen, 2002), the zebrafish is extensively used in genetic and signaling pathway research (Spitsbergen and Kent, 2003). Moreover, their neurophysiological similarities to other vertebrates (Segner, 2009) and robust anxiety-like behavior (Blaser et al., 2010; Blaser and Gerlai, 2006; Egan et al., 2009) have established zebrafish as a useful model of stress-related affective states.

Mounting clinical and experimental evidence indicates that stress strongly affects memory and learning. For example, chronic restraint stress (Yun et al., 2010; Chen et al., 2010; Wright and Conrad, 2005; Hu and Wang, 2006) and exposure to a predator or its odors (Sandi et al., 2005; Diamond et al., 1999, 2006; Morrow et al., 2000; Park et al., 2008; El Hage et al., 2006; Cohen et al., 2009; Kozlovsky et al., 2008; Woodson et al., 2003) impair memory in rodents. Similarly, acute psychological stress affects cognitive functions in humans (Hardison and Purcell, 1959). Although the link between stress and memory is well-recognized, the neurobiology of their interplay remains poorly understood (Kalueff and Nutt, 1996, 2007; Kalueff and Murphy, 2007; Park et al., 2006), and requires novel approaches and new models species.

In addition to stress-related affective behaviors, zebrafish display robust memory and learning abilities, exhibiting strong habituation responses (Wong et al., 2010), olfactory memory (Harden et al., 2006; Braubach et al., 2009) and good learning in tasks using food or the sight of a conspecific as reward (Al-Imari and Gerlai, 2008; Sison and Gerlai, 2010). Zebrafish can perform well in tasks involving cued (Sison and Gerlai, 2010; Williams et al., 2002) or spatial memory (Levin and Chen, 2004; Sison and Gerlai, 2010; Darland and Dowling, 2001), and display overt learned avoidance behavior (Blank et al., 2009; Xu et al., 2007; Barcellos et al., 2010). Possessing robust affective and cognitive phenotypes, adult zebrafish may represent a promising model to study stress–memory interplay. To address this important biomedical problem, the present study focused on examining how acute stress affects zebrafish spatial and cued memory.

2. Materials and methods

2.1. Animals

A total of 60 adult (5–7 month-old, ≈50:50 male:female ratio) wild type, short-fin zebrafish were used in this study. The animals were obtained from a local commercial distributor (50 Fathoms,

* Corresponding author at: Department of Pharmacology, SL-83, Tulane University Medical School, 1430 Tulane Avenue, New Orleans, LA 70112, USA.
Tel.: +1 504 988 3354; fax: +1 504 988 5283.
E-mail address: avkalueff@gmail.com (A.V. Kalueff).

0376-6357/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.beproc.2011.04.004

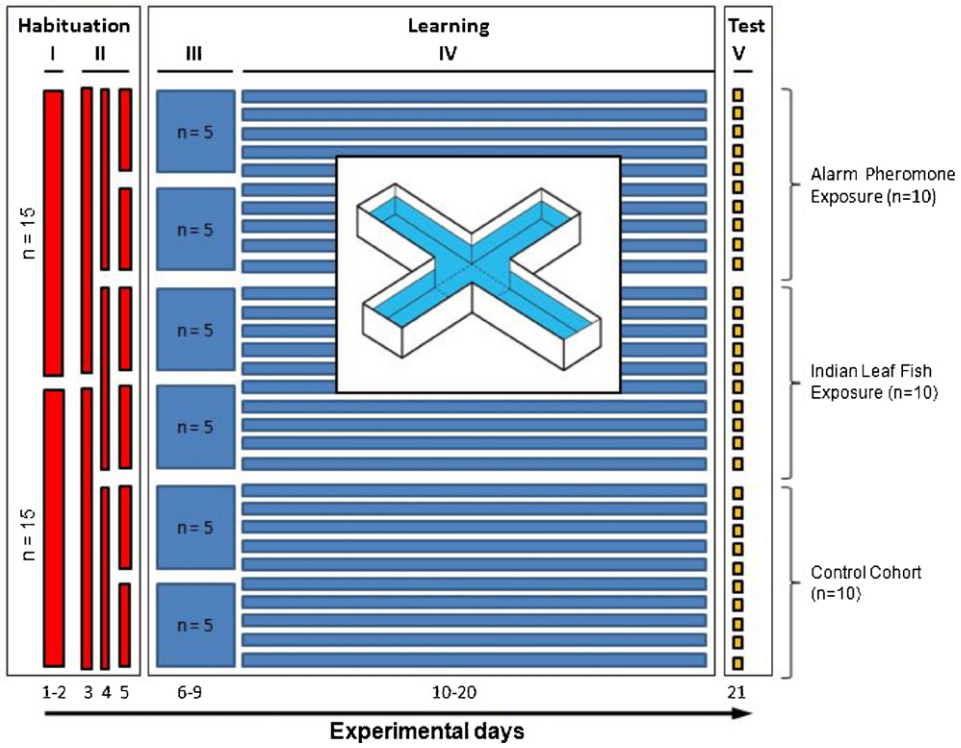

Fig. 1. The flowchart summarizing the experimental design of the present study. The generic diagram illustrates the overall rationale of this study for both cued ($n = 30$) and spatial ($n = 30$) memory tasks used here. Experimental phases: I, initial habituation to the apparatus (2-h trials for days 1–2); II, habituation to the bait (days 3–5); III, group learning (in shoals of 5 fish; days 6–9); IV, individual 6-min daily learning trials (days 10–20); V, the final testing trial (day 21). The plus-maze apparatus used in this study is shown in the inset diagram (only one plus-maze trial per day was used in all experiments of the present study; see Grossman et al., 2011 for methodological details).

Metairie, LA) and were given at least 20 days to acclimate to the animal facility. The fish were housed (15 fish per tank) in 40-L glass tanks (14 cm × 15 cm × 30 cm) at the Tulane University Vivarium. All tanks were filled with filtered facility water, with room and water temperatures maintained at 25–27 °C and water pH at 7.0–7.5. The ceiling-mounted fluorescent lights provided illumination of 1170 ± 67 lux on a 12-h cycle (on 6:00 h; off 18:00 h, consistent with the zebrafish standard of care (Westerfield, 2000)).

2.2. Behavioral testing

The cued memory task used in this study was similar to the protocol recently developed in our laboratory for adult zebrafish (Grossman et al., 2011). Briefly, zebrafish ($n = 30$) in this experiment were required to associate a visible cue (red card placed at different arms) with a food reward (bait). In the spatial memory protocol, zebrafish ($n = 30$) were required to correlate the spatial location of the food reward using external cues (doors, shelves and tables) of the experimental room. One trial was performed per day in all experiments of this study. After 20 days of trials, on the last day of the experiment, all animals were divided into 3 groups ($n = 20$) consisting of controls, alarm pheromone- and predator-exposed zebrafish (see Fig. 1 for details of the experimental design of this study). In the alarm pheromone-exposed group, zebrafish were exposed to alarm pheromone extract ((Egan et al., 2009), see Section 2.3). The predator-exposed group consisted of zebrafish exposed to the natural sympatric predator, Indian leaf fish (*Nandus nandus*). After a standard 6-min acute exposure to the respective stressors, cued or spatial memory was assessed in an unbaited plus-maze trial (Fig. 1).

The test apparatus was a transparent four-armed plus-shaped maze made of Plexiglas (Ezra Scientific, San Antonio, TX) similar to those used in several previous studies (Rodriguez et al., 2002; Salas et al., 1996; Grossman et al., 2011). The arms were 10 cm wide, 10 cm high, 50 cm long, and interconnected by a 10 cm × 10 cm central platform (Fig. 1, inset). The maze was filled with room temperature facility water (25 °C). The fish was initially placed for 30 s in the central compartment, separated from the maze arms by transparent plastic partitions (to avoid bias). These partitions were then raised simultaneously, allowing the fish to explore the four arms freely.

To minimize *procedural* novelty stress, the fish first underwent a series of habituation trials (Fig. 1), which also served to reduce handling stress. To acclimate fish to the plus-maze apparatus, 2-h initial habituation trials were administered on the first two days of the experiment (according to Sison and Gerlai, 2010). During these trials, the fish (in groups of 15) were allowed to freely explore the plus-maze. To minimize acute social isolation stress, zebrafish groups were only gradually reduced in size during the experiment (according to Gleason and Weber, 1977), starting with 15 fish per group on days 1–3 to 10 fish per group on day 4, 5 fish per group on days 6–9, and individual fish testing starting from day 10 (Fig. 1). On days 3–4, the trials lasted 15 min, on days 5–9, the fish were tested in groups of 5 for 6 min. On days 10–20, there were individual-fish 6-min learning trials. To avoid chronic social isolation stress, the animals were returned to their tanks after each plus-maze trial, and housed in their home tanks in groups of 15, as described earlier (Grossman et al., 2011).

Food reward was chosen here as a known efficient reinforcement in zebrafish learning tasks. The need to localize the food position in the maze required a custom insoluble bait (rather than standard Tetramin Tropical flakes; Tetra USA, Blacksburg, VA). For this, a special jelly-like bait was developed and used in the present study. Briefly, 2 g of Tetramin fish food was dissolved in 10 ml of deionized water and vortexed for 2 min, 3 g of Gelatin (Sigma–Aldrich, St. Louis, MO) was then added to this solution and

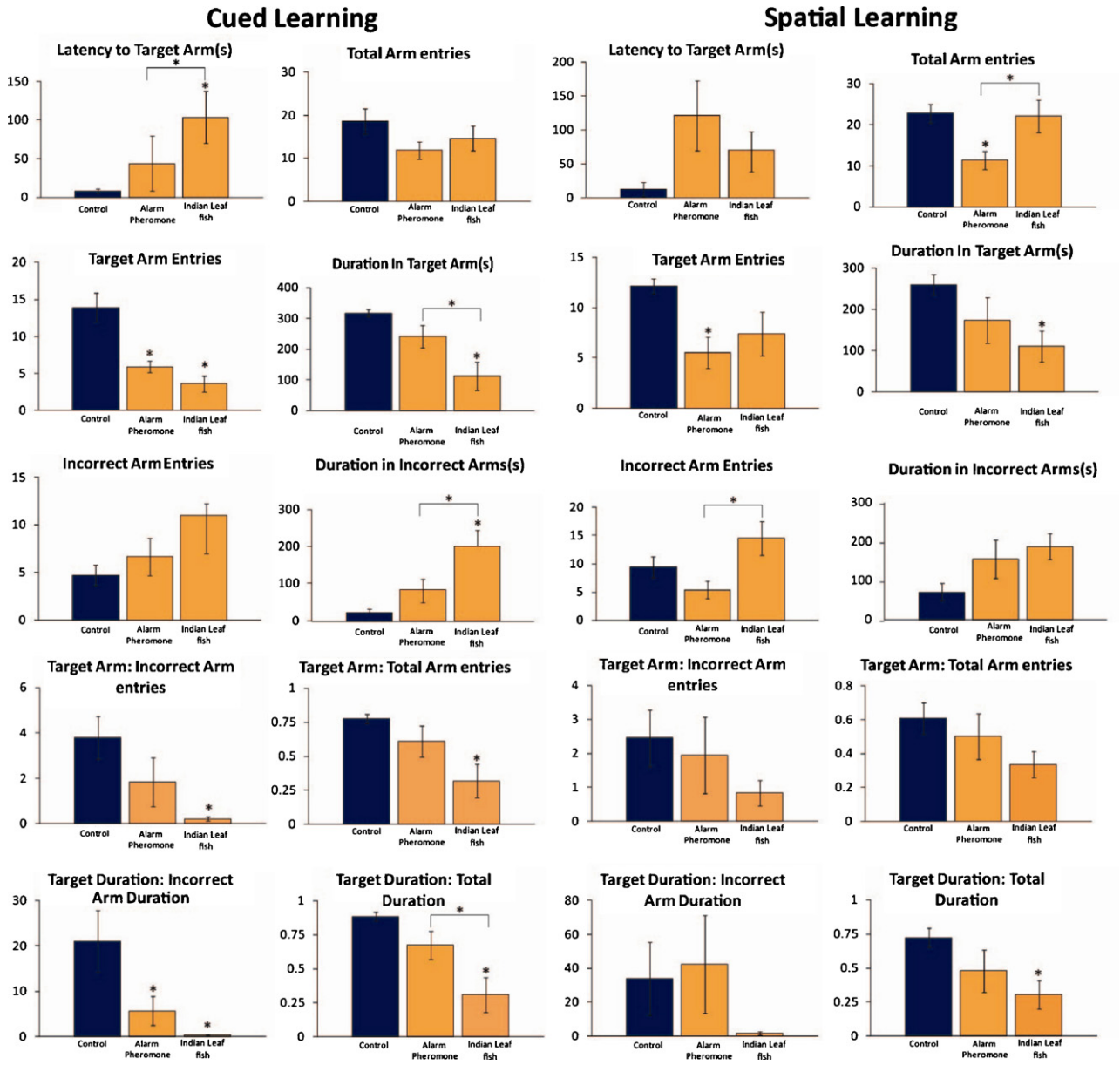

Fig. 2. The effects of acute stressors (6-min alarm pheromone or Indian leaf fish exposure) on cued and spatial memory of zebrafish in the 6-min plus-maze testing trial (day 21; see Fig. 1 for protocol details). Asterisks above data bars indicate significance vs. control group; asterisks above horizontal lines indicate significance between the respective experimental groups. $^{*}p < 0.05$, post-hoc Tukey test for significant ANOVA data ($n = 10$ in each group).

heated to 80 °C for 3 min. The mixture was again vortexed for 2 min, cooled overnight at −20 °C, and used as a bait on subsequent days. Fresh bait was prepared on every second day of this study.

In addition to the apparatus and handling stress, novel food exposure may also confound animal behavioral performance. Therefore, to avoid food neophobia, habituation to the bait (Sison and Gerlai, 2010) was also performed on days 3–5 of the experiment (Fig. 1), in parallel with fish acclimation to the maze apparatus. Since shoaling behavior is innate in zebrafish (Engeszer et al., 2007) and facilitates learning by social transmission (Hall and Suboski, 1995), we started with a 15-fish shoal on day 3, after which the shoal size and trial duration were gradually decreased. Accordingly, the number of baited arms was also gradually reduced, as part of fish habituation to novel food (bait). On day 3, all four arms were baited, on day 4 – only three arms, on day 5 – two arms, and starting from day 6 – only one arm per trial. During individual learning trials (days 10–20), the fish were food-deprived to evoke hunger (as in Smith et al., 2010), and feeding was permitted only during trials, in order to facilitate procedural reinforcement (e.g., Williams et al., 2002; Fig. 1).

The learning trials started after 5 days of habituation to the apparatus (days 1–2) and bait (days 3–5). Following 4 days of group learning (days 6–9), fish were food-deprived for 24 h before beginning individual learning trials, and were only fed in the plus-maze apparatus (Smith et al., 2010; Kim et al., 2010) (Fig. 1). Cumulatively, there were ten 6-min one-arm baited trials over a period of 10 days (days 10–20), followed by a final unbaited *testing* trial on day 21 (Fig. 1).

For the cued memory task, a red plastic 10 cm × 10 cm cue card (chosen because zebrafish can see and react to the red color (Brockerhoff et al., 1997)) was placed adjacent to the reward arm. During the trials, the baited arm location (denoted by the red card) was randomly changed to prevent bias. Since spatial learning is robust in fish species (Salas et al., 2008), it was used in our zebrafish study. The location of the reward in this paradigm was not marked by an intra-maze visible cue, but was instead identified based upon

external visual cues surrounding the plus-maze (e.g., table, door and fluorescent lights). For both memory tests, there were four 5-fish (days 6–9) and ten single-fish baited trials (days 10–20), followed by a final unbaited *testing* trial on day 21 (Fig. 1).

2.3. Stressors

Alarm pheromone and Indian leaf fish were used here as ecologically relevant stressors, both known to strongly affect zebrafish behavior. These two stressors evoke consistent anxiogenic responses in zebrafish tested by several different groups (Jesuthasan and Mathuru, 2008; Speedie and Gerlai, 2008; Gerlai et al., 2009; Ahmed et al., 2011), and are routinely used in our laboratory to evoke anxiety-like behavioral and endocrine (cortisol) responses in zebrafish (Egan et al., 2009; Cachat et al., 2010a; Stewart et al., 2010). Alarm pheromone is secreted by damaged epidermal cells, eliciting an innate fear in nearby fish (Hall and Suboski, 1995; Rehnberg et al., 1989). The extraction procedure was adapted from previous studies (Speedie and Gerlai, 2008; Egan et al., 2009) and performed using a surgical blade in a Petri dish kept on ice. Fifteen shallow cuts were made on each side of the euthanized fish and washed with 10 ml of distilled water. Shallow cuts were made to prevent contamination by blood, and the extract was immediately collected. Zebrafish were then individually transferred to the 3-L exposure beaker, exposed to 7 ml of the alarm pheromone extract for 6 min, and placed individually into the plus-maze for the final (testing) trial (Cachat et al., 2010b). Control fish were exposed to a 3-L beaker containing pheromone-free facility water.

The Indian leaf fish is a natural sympatric predator of zebrafish (Talwar and Jhingran, 1991), eliciting their strong fear/anxiety-like responses (Gerlai et al., 2009; Bass and Gerlai, 2008; Egan et al., 2009). The zebrafish and the Indian leaf fish were housed in separate tanks, not visible to one another. The Indian leaf fish was adequately fed, and as a nocturnal feeder, did not show predatory attacks during the exposure. Precautionary measures were taken during the procedure to prevent harm to the experimental animals. Zebrafish were individually placed in a 3-L exposure beaker with an unrestrained well-fed predator for 6 min, followed by the plus-maze testing trial (Fig. 1). Control fish were exposed to an empty (predator-free) 3-L exposure beaker.

2.4. Behavioral analyses

The zebrafish behavior during the final testing trial was analyzed by Ethovision XT7 (Noldus IT, Wageningen, Netherlands). The plus-maze was divided into right, left, bottom and top arenas. For each analysis, one of the four arms was denoted as the target (correct) arm, and the other three were grouped as incorrect arms. Behavioral quantification was performed for the following endpoints: latency to the target arm (s), the number of target arm, incorrect arm and total arm entries, duration in the target or incorrect arms (s), as well as velocity (m/s), distance moved (m) and swimming duration (s). To assess the efficacy of zebrafish memory, we compared the number of target vs. incorrect arm entries and time spent demonstrated by the control groups in the study (after 14 days of learning trials) on the final testing trial (day 21, Fig. 1). We also examined the ratios of target:incorrect and target:total arm entries and time spent on the testing trial for all three experimental groups.

2.5. Statistics

Behavioral data were analyzed statistically using SPSS 18.0 by one-way ANOVA tests (factor: group/stress treatment), followed by a Tukey post-hoc test for significant ANOVA data. Target vs. incorrect arm behavior of the control groups in both memory tests was analyzed using the paired Wilcoxon–Mann–Whitney *U*-test. All results were expressed as mean ± S.E.M. and significance was set at $p < 0.05$.

3. Results

To confirm the efficiency of the learning trials, we compared the target vs. incorrect arm behaviors in the final (testing) trial in the control fish cohorts. In the cued memory task, control fish showed significantly longer duration in the target arm (318 ± 11 s vs. 23 ± 7 s) and made more correct entries (14 ± 2 vs. 5 ± 1, $p < 0.005$, *U*-test), confirming very good zebrafish learning and memory in this study. Similarly, in the spatial memory task, control fish spent significantly more time in the target arm (260 ± 25 s vs. 70 ± 22, $p < 0.01$, *U*-test), although the number of correct entries (12 ± 0.8 vs. 9 ± 1.8, NS) did not reach significance.

We next assessed the effects of acute stressors on zebrafish cued memory. One-way ANOVA revealed a significant group effect for the latency to the target arm ($F_{(2,29)} = 9.3$, $p < 0.001$), with the predator-exposed animals showing significantly longer latency to the target arm, compared to both the control and alarm pheromone-exposed groups (Fig. 2). One-way ANOVA also found a significant group effect for the number of target arm entries ($F_{(2,29)} = 15.8$, $p < 0.00001$), with both alarm pheromone- and predator-exposed fish showing significantly fewer target arm entries (Fig. 2). The predator-exposed group spent less time in the target arm compared to the other two groups in the cued memory task. There were also significant group effects for duration in the target arm ($F_{(2,29)} = 8.9$, $p < 0.001$) and incorrect arms ($F_{(2,29)} = 8.5$, $p < 0.001$), but not for total or incorrect arm entries (Fig. 2). Overall, predator-exposed fish spent less time in target arm, and more time in incorrect arms, compared to both control and alarm pheromone-exposed groups. Analysis of the target:incorrect and target:total ratios showed that the predator stress strongly affected fish behavior in this task, significantly reducing both ratios for the number of entries and time spent endpoints. Alarm pheromone exposure produced similar effects on target:incorrect arm entries ratio, but did not significantly affect other ratios (Fig. 2). There were no significant group effects on zebrafish velocity, distance traveled or swimming duration in this paradigm (Fig. 3).

In the spatial memory task, one-way ANOVA testing revealed significant group effects for the number of target ($F_{(2,29)} = 4.5$, $p < 0.05$), incorrect ($F_{(2,29)} = 3.8$, $p < 0.05$) and total arm entries ($F_{(2,29)} = 4.7$, $p < 0.05$), as well as for the duration in target arm ($F_{(2,29)} = 3.3$, $p < 0.05$), but not for the latency to target arm, duration in incorrect arms, target:incorrect arm entries or duration ratios, and target:total arm entries ratio (Fig. 2). Similarly, there were no group effects for velocity and distance traveled, but a significant group effect on swimming duration ($F_{(2,29)} = 4.7$, $p < 0.05$; Fig. 3).

Overall, the alarm pheromone-exposed fish displayed fewer target and total arm entries, and spent more time frozen, compared to the other two groups (Figs. 2 and 3). The predator-exposed group spent less time in the target arm, compared to control, and significantly more time in the incorrect arm vs. the alarm pheromone-exposed fish (Fig. 2). The alarm pheromone-exposed group spent less time swimming, compared to controls, whereas the predator-exposed fish showed a lower target:incorrect arm duration ratio in this paradigm.

4. Discussion

Stress is known to affect cognitive functions in humans (Cousijn et al., 2010; Daversa, 2010; Patil et al., 1995), and may impair learning and memory in various animal models (Zhang et al., 2003; Ohl and Fuchs, 1999), including mice (Grootendorst et al., 2001) and

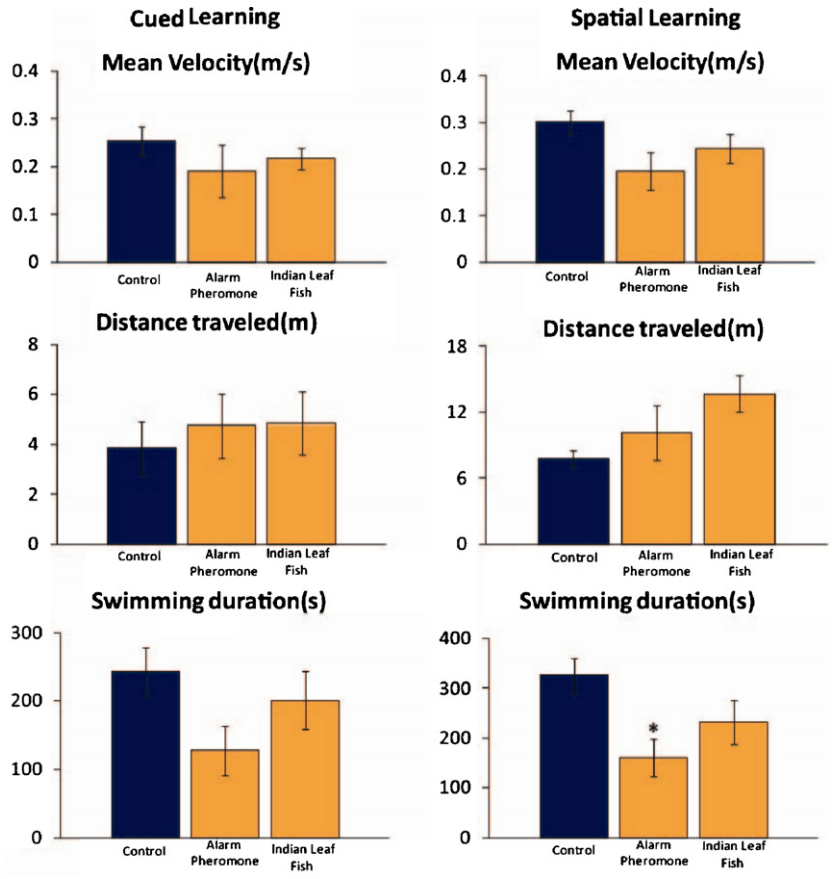

Fig. 3. Effects of alarm pheromone and Indian leaf fish exposure on motor behavior in cued and spatial memory tests in zebrafish exposed to the 6-min plus-maze testing trial (day 21). Asterisks above data bars indicate significance vs. controls. $^{*}p < 0.05$, post-hoc Tukey test for significant ANOVA data ($n = 10$ in each group).

rats (Park et al., 2008; Diamond et al., 1999, 2006; Sandi et al., 2005; Cohen et al., 2009). Recently, the effects of stress have also been examined in zebrafish, using predator exposure, electric shock or alarm pheromone as stressors (Barcellos et al., 2007; Blank et al., 2009; Gerlai et al., 2009; Jesuthasan and Mathuru, 2008; Pradel et al., 2000; Wong et al., 2010). The impact of chronic unpredictable mild stress has also been studied in zebrafish anxiety and cognitive tests (Piato et al., 2010). Although good learning and memory abilities have been demonstrated in various zebrafish studies (Sison and Gerlai, 2010; Al-Imari and Gerlai, 2008; Blank et al., 2009; Colwill et al., 2005), the effects of acute stress on their memory have only recently been explored (Piato et al., 2010; de Castro et al., 2009). This study is the first report specifically focusing on the impact of acute stress on performance in memory tests in adult zebrafish.

Overall, our experiments showed that the target arm preference (duration and target:incorrect ratios) was significantly affected by acute stress in the cued memory task, suggesting that acute psychological stressors may extend the amount of time required to associate a learned cue with the food location. Likewise, acute stress may also cause overall confusion in regards to locating the food, as demonstrated by a significant decrease in target arm entries, without overt variations in velocity or distance traveled during the experimental trial (Figs. 2 and 3).

In the spatial memory task, target, incorrect and total arm entries were also significantly affected by the predator stress. Unlike the cued memory task, spatial learning develops as zebrafish associate immediate environmental landmarks with the location of a food reward. Alarm pheromone-exposed fish made less target or incorrect arm entries, and spent significantly less time swimming (Figs. 2 and 3), whereas the predator-exposed fish initiated more incorrect arm entries and spent less time in the target arm. The overall decrease in the target arm preference suggests that predator exposure strongly affected zebrafish performance in the spatial memory test (Fig. 2).

One logical explanation for acute stress-evoked impaired performance in zebrafish here may be hyper-arousal, known to affect memory according to the Yerkes–Dodson law. In line with this, acute pro-arousal stressors such as alarm pheromone or anxiogenic drugs pentylenetetrazole (PTZ) and caffeine impaired zebrafish spatial working memory (habituation) in our previous studies (Wong et al., 2010). In addition to these effects on spatial working memory, PTZ also impaired passive avoidance learning in zebrafish (Lee et al., 2010; Kim et al., 2009). Together with our present data, these findings indicate that zebrafish may be a sensitive model to study the effects of various experimental stressors on cognitive functions.

In addition to memory modulation *per se*, several alternative possibilities include sensorimotor impairments evoked by acute stressors, as well as reduced foraging motivation, increased escape-seeking, or both. However, if the stressors reduced foraging motivation, the cued memory task performance (based on association of cue with food reward) would have been more affected compared to the spatial memory task (based on spatial mapping). If stress increased the avoidance motivation in this study, then spatial memory task performance would have been affected more strongly, compared to the cued memory test. In contrast, our results indicate that the two stressors used here affected performance of zebrafish in both memory tasks equally well, based on the number of significantly affected endpoints, especially those related to target arm preference (Fig. 2). This suggests that a single motivation was

unlikely to underlie the observed phenotypes in this study. An alternative possibility could be simultaneous modulation of these two motivations. However, increased avoidance should result in more total entries and, most likely, higher velocity and longer distance traveled. Figs. 2 and 3 show that stress did not cause this response in the present study, therefore negating the possibility that two motivations are simultaneously affected by stress. Finally, the fact that stress exposure did not alter the general motor characteristics of zebrafish swimming (Fig. 3) implies the lack of sensorimotor impairment in zebrafish in these tasks. Taken together, this supports the possibility that memory impairment evoked by acute stress exposure was the major cause of the observed behavioral phenotypes, in line with stress-evoked memory modulation in clinical and rodent findings (Kalueff and Nutt, 1996, 2007; Kalueff and Murphy, 2007).

Taken together, our results support the need for further research testing the effects of experimental stressors on various forms of memory in adult zebrafish. In addition to the approach used here, other types of stressors (including both acute and chronic stress), different forms of memory and additional cognitive tasks may be applied. Likewise, the effects of other experimental challenges (including mnemotropic drugs, strain differences, sex, age or genetic manipulation) on zebrafish memory may be examined using our protocol (see, for example, the effects of piracetam on improving zebrafish memory in the cued memory task (Grossman et al., 2011)). Additionally, the plus-maze memory paradigm may be useful in identifying and characterizing the genetic and physiological factors, as well as the circuits and signaling pathways, involved in cognitive impairment. Overall, the efficacy of acute stressors in their modulation of zebrafish performance in memory tasks indicates the relevance of this paradigm to modeling the interplay between memory and stress.

Acknowledgements

The authors thank M. Hook, K. Rhymes, K. Chang, E. Kyzar, I. Bruce, M. Greenberg and M. Caffery for their help with this project. The study was supported by Tulane University, Newcomb Fellows Grant, Zebrafish Neuroscience Research Consortium (ZNRC) and the Louisiana Board of Regents NSF-sponsored P-fund Grant.

References

Ahmed, O., Seguin, D., Gerlai, R., 2011. An automated predator avoidance task in zebrafish. Behav. Brain Res. 216, 166–171.
Al-Imari, L., Gerlai, R., 2008. Sight of conspecifics as reward in associative learning in zebrafish (*Danio rerio*). Behav. Brain Res. 189, 216–219.
Barcellos, L.J.G., Ritter, F., Kreutz, L.C., Cericato, L., 2010. Can zebrafish *Danio rerio* learn about predation risk? The effect of a previous experience on the cortisol response in subsequent encounters with a predator. J. Fish Biol. 76, 1032–1038.
Barcellos, L.J.G., Ritter, F., Kreutz, L.C., Quevedo, R.M., da Silva, L.B., Bedin, A.C., Finco, J., Cericato, L., 2007. Whole-body cortisol increases after direct and visual contact with a predator in zebrafish, *Danio rerio*. Aquaculture 272, 774–778.
Bass, S.L., Gerlai, R., 2008. Zebrafish (*Danio rerio*) responds differentially to stimulus fish: the effects of sympatric and allopatric predators and harmless fish. Behav. Brain Res. 186, 107–117.
Blank, M., Guerim, L.D., Cordeiro, R.F., Vianna, M.R., 2009. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. Neurobiol. Learn. Mem. 92, 529–534.
Blaser, R., Gerlai, R., 2006. Behavioral phenotyping in zebrafish: comparison of three behavioral quantification methods. Behav. Res. Methods 38, 456–469.
Blaser, R.E., Chadwick, L., McGinnis, G.C., 2010. Behavioral measures of anxiety in zebrafish (*Danio rerio*). Behav. Brain Res. 208, 56–62.
Braubach, O.R., Wood, H.D., Gadbois, S., Fine, A., Croll, R.P., 2009. Olfactory conditioning in the zebrafish (*Danio rerio*). Behav. Brain Res. 198, 190–198.
Brockerhoff, S.E., Hurley, J.B., Niemi, G.A., Dowling, J.E., 1997. A new form of inherited red-blindness identified in zebrafish. J. Neurosci. 17, 4236–4242.
Cachat, J., Stewart, A., Grossman, L., Gaikwad, S., Kadri, F., Min Chung, K., Wu, N., Wong, K., Roy, S., Suciu, C., Goodspeed, J., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Tan, J., Denmark, A., Gilder, T., Kyzar, E., DiLeo, J., Frank, K., Chang, K., Utterback, E., Hart, P., Kalueff, A., 2010a. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat. Protoc. 5, 1786–1799.
Cachat, J., Elegante, C.P., Bartels, M., Elkhayat, B., Hart, S., Tien, P., Tien, A., Mohnot, D.B.E., Laffoon, S., Stewart, A., Gaikwad, A., Wong, S., Haymore, K., Kalueff, W.A., 2010b. Modeling Stress and Anxiety in Zebrafish. In: Kalueff, C.J.A. (Ed.), Zebrafish Models in Neurobehavioral Research. Humana Press, New Orleans.
Chen, Y., Mao, Y., Zhou, D., Hu, X., Wang, J., Ma, Y., 2010. Environmental enrichment and chronic restraint stress in ICR mice: effects on prepulse inhibition of startle and Y-maze spatial recognition memory. Behav. Brain Res. 212, 49–55.
Cohen, H., Liberzon, I., Richter-Levin, G., 2009. Exposure to extreme stress impairs contextual odour discrimination in an animal model of PTSD. Int. J. Neuropsychopharmacol. 12, 291–303.
Colwill, R.M., Raymond, M.P., Ferreira, L., Escudero, H., 2005. Visual discrimination learning in zebrafish (*Danio rerio*). Behav. Process. 70, 19–31.
Cousijn, H., Rijpkema, M., Qin, S., van Marle, H.J., Franke, B., Hermans, E.J., van Wingen, G., Fernandez, G., 2010. Acute stress modulates genotype effects on amygdala processing in humans. Proc. Natl. Acad. Sci. U.S.A. 107, 9867–9872.
Darland, T., Dowling, J.E., 2001. Behavioral screening for cocaine sensitivity in mutagenized zebrafish. Proc. Natl. Acad. Sci. U.S.A. 98, 11691–11696.
Daversa, M.T., 2010. Early environmental predictors of the affective and interpersonal constructs of psychopathy. Int. J. Offender Ther. Comp. Criminol. 54, 6–21.
de Castro, M.R., Lima, J.V., de Freitas, D.P., Valente Rde, S., Dummer, N.S., de Aguiar, R.B., dos Santos, L.C., Marins, L.F., Geracitano, L.A., Monserrat, J.M., Barros, D.M., 2009. Behavioral and neurotoxic effects of arsenic exposure in zebrafish (*Danio rerio*, Teleostei: Cyprinidae). Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 150, 337–342.
Diamond, D.M., Campbell, A.M., Park, C.R., Woodson, J.C., Conrad, C.D., Bachstetter, A.D., Mervis, R.F., 2006. Influence of predator stress on the consolidation versus retrieval of long-term spatial memory and hippocampal spinogenesis. Hippocampus 16, 571–576.
Diamond, D.M., Park, C.R., Heman, K.L., Rose, G.M., 1999. Exposing rats to a predator impairs spatial working memory in the radial arm water maze. Hippocampus 9, 542–552.
Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhayat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., Mohnot, S., Beeson, E., Glasgow, E., Amri, H., Zukowska, Z., Kalueff, A.V., 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav. Brain Res. 205, 38–44.
El Hage, W., Griebel, G., Belzung, C., 2006. Long-term impaired memory following predatory stress in mice. Physiol. Behav. 87, 45–50.
Engeszer, R.E., Patterson, L.B., Rao, A.A., Parichy, D.M., 2007. Zebrafish in the wild: a review of natural history and new notes from the field. Zebrafish 4, 21–40.
Gerlai, R., Fernandes, Y., Pereira, T., 2009. Zebrafish (*Danio rerio*) responds to the animated image of a predator: towards the development of an automated aversive task. Behav. Brain Res. 201, 318–324.
Gerlai, R., Lee, V., Blaser, R., 2006. Effects of acute and chronic ethanol exposure on the behavior of adult zebrafish (*Danio rerio*). Pharmacol. Biochem. Behav. 85, 752–761.
Gleason, P.E., Weber, P.G., 1977. Effect of group size on avoidance learning in zebrafish, *Brachydanio rerio* (Pisces: Cyprinidae). Anim. Learn. Behav. 5, 213–216.
Grootendorst, J., de Kloet, E.R., Vossen, C., Dalm, S., Oitzl, M.S., 2001. Repeated exposure to rats has persistent genotype-dependent effects on learning and locomotor activity of apolipoprotein E knockout and C57Bl/6 mice. Behav. Brain Res. 125, 249–259.
Grossman, L., Stewart, A., Gaikwad, S., Utterback, E., Wu, N., Dileo, J., Frank, J., Hart, P., Howard, H., Kalueff, A.V., 2011. Effects of piracetam on behavior and learning in adult zebrafish. Brain Res. Bull. 85, 58–63.
Grunwald, D.J., Eisen, J.S., 2002. Headwaters of the zebrafish – emergence of a new model vertebrate. Nat. Rev. Genet. 3, 717–724.
Hall, D., Suboski, M.D., 1995. Visual and olfactory stimuli in learned release of alarm reactions by zebra danio fish (*Brachydanio rerio*). Neurobiol. Learn. Mem. 63, 229–240.
Harden, M.V., Newton, L.A., Lloyd, R.C., Whitlock, K.E., 2006. Olfactory imprinting is correlated with changes in gene expression in the olfactory epithelia of the zebrafish. J. Neurobiol. 66, 1452–1466.
Hardison, J., Purcell, K., 1959. The effects of psychological stress as a function of need and cognitive control. J. Pers. 27, 250–258.
Hu, X.Y., Wang, D.X., 2006. Effects of lidocaine on learning and memory dysfunction as well neuropathologic change induced by chronic stress: experiment with mice. Zhonghua Yi Xue Za Zhi 86, 3335–3339.
Jesuthasan, S.J., Mathuru, A.S., 2008. The alarm response in zebrafish: innate fear in a vertebrate genetic model. J. Neurogenet. 22, 211–228.
Kalueff, A., Nutt, D.J., 1996. Role of GABA in memory and anxiety. Depress. Anxiety 4, 100–110.
Kalueff, A.V., Murphy, D.L., 2007. The importance of cognitive phenotypes in experimental modeling of animal anxiety and depression. Neural. Plast. 2007, 52087.
Kalueff, A.V., Nutt, D.J., 2007. Role of GABA in anxiety and depression. Depress. Anxiety 24, 495–517.
Kim, Y., Lee, Y., Lee, H., Jung, M., Lee, C., 2009. Impaired avoidance learning and increased hsp70 mRNA expression in pentylenetetrazol-treated zebrafish. Anim. Cells Syst. 13, 275–281.
Kim, Y.H., Lee, Y., Kim, D., Jung, M.W., Lee, C.J., 2010. Scopolamine-induced learning impairment reversed by physostigmine in zebrafish. Neurosci. Res. 67, 156–161.
Kozlovsky, N., Matar, M.A., Kaplan, Z., Kotler, M., Zohar, J., Cohen, H., 2008. The immediate early gene Arc is associated with behavioral resilience to stress exposure in an animal model of posttraumatic stress disorder. Eur. Neuropsychopharmacol. 18, 107–116.

Lee, Y., Kim, D., Kim, Y.H., Lee, H., Lee, C.J., 2010. Improvement of pentylenetetrazol-induced learning deficits by valproic acid in the adult zebrafish. Eur. J. Pharmacol..

Levin, E.D., Chen, E., 2004. Nicotinic involvement in memory function in zebrafish. Neurotoxicol. Teratol. 26, 731–735.

Morrow, B.A., Roth, R.H., Elsworth, J.D., 2000. TMT, a predator odor, elevates mesoprefrontal dopamine metabolic activity and disrupts short-term working memory in the rat. Brain Res. Bull. 52, 519–523.

Ohl, F., Fuchs, E., 1999. Differential effects of chronic stress on memory processes in the tree shrew. Brain Res. Cogn. Brain Res. 7, 379–387.

Park, C.R., Campbell, A.M., Woodson, J.C., Smith, T.P., Fleshner, M., Diamond, D.M., 2006. Permissive influence of stress in the expression of a U-shaped relationship between serum corticosterone levels and spatial memory errors in rats. Dose Response 4, 55–74.

Park, C.R., Zoladz, P.R., Conrad, C.D., Fleshner, M., Diamond, D.M., 2008. Acute predator stress impairs the consolidation and retrieval of hippocampus-dependent memory in male and female rats. Learn. Mem. 15, 271–280.

Patil, P.G., Apfelbaum, J.L., Zacny, J.P., 1995. Effects of a cold-water stressor on psychomotor and cognitive functioning in humans. Physiol. Behav. 58, 1281–1286.

Piato, A.L., Capiotti, K.M., Tamborski, A.R., Oses, J.P., Barcellos, L.J., Bogo, M.R., Lara, D.R., Vianna, M.R., Bonan, C.D., 2010. Unpredictable chronic stress model in zebrafish (*Danio rerio*): behavioral and physiological responses. Prog. Neuropsychopharmacol. Biol. Psychiatry.

Pradel, G., Schmidt, R., Schachner, M., 2000. Involvement of L1.1 in memory consolidation after active avoidance conditioning in zebrafish. J. Neurobiol. 43, 389–403.

Rehnberg, B.G., Bates, E.H., Smith, R.J., Sloley, B.D., Richardson, J.S., 1989. Brain benzodiazepine receptors in fathead minnows and the behavioral response to alarm pheromone. Pharmacol. Biochem. Behav. 33, 435–442.

Rodriguez, F., Lopez, J.C., Vargas, J.P., Broglio, C., Gomez, Y., Salas, C., 2002. Spatial memory and hippocampal pallium through vertebrate evolution: insights from reptiles and teleost fish. Brain Res. Bull. 57, 499–503.

Salas, C., Broglio, C., Durán, E., Gómez, A., Rodríguez, F., 2008. Spatial learning in fish. In: John, H.B. (Ed.), Learning and Memory: A Comprehensive Reference. Academic Press, Oxford.

Salas, C., Rodriguez, F., Vargas, J.P., Duran, E., Torres, B., 1996. Spatial learning and memory deficits after telencephalic ablation in goldfish trained in place and turn maze procedures. Behav. Neurosci. 110, 965–980.

Sandi, C., Woodson, J.C., Haynes, V.F., Park, C.R., Touyarot, K., Lopez-Fernandez, M.A., Venero, C., Diamond, D.M., 2005. Acute stress-induced impairment of spatial memory is associated with decreased expression of neural cell adhesion molecule in the hippocampus and prefrontal cortex. Biol. Psychiatry 57, 856–864.

Segner, H., 2009. Zebrafish (*Danio rerio*) as a model organism for investigating endocrine disruption. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 149, 187–195.

Sison, M., Gerlai, R., 2010. Associative learning in zebrafish (*Danio rerio*) in the plus maze. Behav. Brain Res. 207, 99–104.

Smith, L.E., Carvan 3rd, M.J., Dellinger, J.A., Ghorai, J.K., White, D.B., Williams, F.E., Weber, D.N., 2010. Developmental selenomethionine and methylmercury exposures affect zebrafish learning. Neurotoxicol. Teratol. 32, 246–255.

Speedie, N., Gerlai, R., 2008. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). Behav. Brain Res. 188, 168–177.

Spitsbergen, J.M., Kent, M.L., 2003. The state of the art of the zebrafish model for toxicology and toxicologic pathology research—advantages and current limitations. Toxicol. Pathol. (Suppl 31), 62–87.

Stewart, A., Kadri, F., DiLeo, J., Chung, K., Cachat, J., Goodspeed, J., Suciu, C., Roy, S., Gaikwad, S., Wong, K., Elegante, M., Elkhayat, S., Wu, N., Gilder, T., Tien, D., Kalueff, A.V., 2010. The developing utility of zebrafish in modeling neurobehavioral disorders. Int. J. Comp. Psychol. 23, 104–121.

Talwar, P.K., Jhingran, A.G., 1991. Inland Fishes of India and Adjacent Countries. CRC Press.

Westerfield, M., 2000. The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*).

Williams, F.E., White, D., Messer, W.S., 2002. A simple spatial alternation task for assessing memory function in zebrafish. Behav. Process. 58, 125–132.

Wong, K., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Roy, S., Goodspeed, J., Suciu, C., Tan, J., Grimes, C., Chung, A., Rosenberg, M., Gaikwad, S., Denmark, A., Jackson, A., Kadri, F., Chung, K.M., Stewart, A., Gilder, T., Beeson, E., Zapolsky, I., Wu, N., Cachat, J., Kalueff, A.V., 2010. Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). Behav. Brain Res. 208, 450–457.

Woodson, J.C., Macintosh, D., Fleshner, M., Diamond, D.M., 2003. Emotion-induced amnesia in rats: working memory-specific impairment, corticosterone-memory correlation, and fear versus arousal effects on memory. Learn. Mem. 10, 326–336.

Wright, R.L., Conrad, C.D., 2005. Chronic stress leaves novelty-seeking behavior intact while impairing spatial recognition memory in the Y-maze. Stress 8, 151–154.

Xu, X., Scott-Scheiern, T., Kempker, L., Simons, K., 2007. Active avoidance conditioning in zebrafish (*Danio rerio*). Neurobiol. Learn. Mem. 87, 72–77.

Yun, J., Koike, H., Ibi, D., Toth, E., Mizoguchi, H., Nitta, A., Yoneyama, M., Ogita, K., Yoneda, Y., Nabeshima, T., Nagai, T., Yamada, K., 2010. Chronic restraint stress impairs neurogenesis and hippocampus-dependent fear memory in mice: possible involvement of a brain-specific transcription factor Npas4. J. Neurochem..

Zhang, Y.M., Yang, Q., Xu, C.T., Li, K.S., Li, W.Q., 2003. Effects of phenytoin on morphology and structure of hippocampal CA3 pyramidal neurons of rats in chronic stress. Acta Pharmacol. Sin. 24, 403–407.