



## Taurine modulates the stress response in zebrafish

Nathana J. Mezzomo<sup>a,c,\*</sup>, Barbara D. Fontana<sup>a,b</sup>, Talise E. Müller<sup>a,b</sup>, Tâmie Duarte<sup>a,b</sup>,  
Vanessa A. Quadros<sup>a,b</sup>, Julia Canzian<sup>a</sup>, Aline Pompermaier<sup>e</sup>, Suelen M. Soares<sup>c</sup>, Gessi Koakoski<sup>e</sup>,  
Vania L. Loro<sup>b</sup>, Denis B. Rosemberg<sup>a,b,d</sup>, Leonardo J.G. Barcellos<sup>c,e,f</sup>

<sup>a</sup> Laboratory of Experimental Neuropsychobiology, Department of Biochemistry and Molecular Biology, Natural and Exact Sciences Center, Federal University of Santa Maria, 1000 Roraima Avenue, Santa Maria, RS 97105-900, Brazil

<sup>b</sup> Graduate Program in Biological Sciences: Toxicological Biochemistry, Federal University of Santa Maria, 1000 Roraima Avenue, Santa Maria, RS 97105-900, Brazil

<sup>c</sup> Graduate Program in Pharmacology, Federal University of Santa Maria, 1000 Roraima Avenue, Santa Maria, RS 97105-900, Brazil

<sup>d</sup> The International Zebrafish Neuroscience Research Consortium (ZNRC), 309 Palmer Court, Slidell, LA 70458, USA

<sup>e</sup> Graduate Program in Bio-Experimentation, University of Passo Fundo (UPF), BR 285, Passo Fundo, RS 99052-900, Brazil

<sup>f</sup> Graduate Program in Environmental Sciences, University of Passo Fundo (UPF), BR 285, Passo Fundo, RS 99052-900, Brazil

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### ABSTRACT

The zebrafish (*Danio rerio*) is used as an emergent model organism to investigate the behavioral and physiological responses to stress. The anxiolytic-like effects of taurine in zebrafish support the existence of different mechanisms of action, which can play a role in preventing stress-related disorders (i.e., modulation of GABA<sub>A</sub>, strychnine-sensitive glycine, and NMDA receptors, as well as antioxidant properties). Herein, we investigate whether taurine modulates some behavioral and biochemical responses in zebrafish acutely submitted to chemical and mechanical stressors. We pretreated zebrafish for 1 h in beakers at 42, 150, and 400 mg/L taurine. Fish were later acutely exposed to a chemical stressor (conspecific alarm substance) or to a mechanical stressor (net chasing), which elicits escaping responses and aversive behaviors. Locomotion, exploration, and defensive-like behaviors were measured using the novel tank and the light–dark tests. Biochemical (brain oxidative stress-related parameters) and whole-body cortisol levels were also quantified. We showed that taurine prevents anxiety/fear-like behaviors and protein carbonylation and dampens the cortisol response following acute stress in zebrafish. In summary, our results demonstrate a protective role of taurine against stress-induced behavioral and biochemical changes, thereby reinforcing the growing utility of zebrafish models to investigate the neuroprotective actions of taurine in vertebrates.

### 1. Introduction

The zebrafish (*Danio rerio*) is widely used as a model organism to investigate behavioral and neurochemical aspects of stress-related neuropsychiatric disorders (Brennan, 2011; Fontana et al., 2019; Kalueff et al., 2014; Mezzomo et al., 2018; Norton and Bally-Cuif, 2010; Norton, 2013). This species shows a high degree of genetic and physiological conservation, with various brain structures having homologous functions when compared with the mammalian counterparts (Randlett et al., 2015; Ullmann et al., 2010). The lateral pallium of the telencephalon is responsible for memory processing, while the dorsal habenula controls anxiety/fear responses (Mathuru and Jesuthasan, 2013). These brain structures are analogous to the hippocampus and amygdala, respectively (Agetsuma et al., 2010; Perathoner et al., 2016). Moreover, the zebrafish expresses all major neurotransmitter systems

(e.g., dopaminergic, serotonergic, cholinergic, and noradrenergic) described in mammals (Agostini et al., 2018; Kasthuber et al., 2010; Lillesaar et al., 2007; Schweitzer and Driever, 2009). Zebrafish displays complex cognitive processing and decision-making strategies, showing high sensitivity to pharmacological agents that modulate behavioral functions (Oliveira, 2013; Parker et al., 2012; Sison et al., 2006). This species shows robust aversive responses (e.g., anxiety/fear-like behaviors), when exposed to stressors, which are pharmacologically sensitive to antistress agents (Gerlai, 2010; Maximino et al., 2010; Steenbergen et al., 2011). When studying anxiety/fear responses resulting from abnormal stress-related physiology, stress hormone levels and oxidative stress-related parameters can serve as valuable tools to parallel with behavioral observations (Alsop and Vijayan, 2009; Holsboer, 2000; Moreno-Peral et al., 2014; Newport and Nemeroff, 2000; Walker et al., 2013).

\* Corresponding author at: Graduate Program in Pharmacology, Federal University of Santa Maria, 1000 Roraima Avenue, Santa Maria, RS 97105-900, Brazil.  
E-mail address: [nathanajamillemezzomo@gmail.com](mailto:nathanajamillemezzomo@gmail.com) (N.J. Mezzomo).

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The hypothalamic–pituitary–interrenal (HPI) axis coordinates the stress responses in zebrafish, which involves a cascade of hormones, starting from corticotropin-releasing factor (CRF) to adrenocorticotropic hormone (ACTH) and cortisol, released from the interrenal cells (adrenal gland homolog) (Fuzzen et al., 2010; Ghisleni et al., 2012; Tran et al., 2014). Cortisol binds to the glucocorticoid receptor that regulates the transcription of target genes related to glucose metabolism, immune function, and behavior (Bury and Sturm, 2007; Vijayan et al., 2016). Numerous reports show the growing utility of zebrafish models for assessing behavioral, neurochemical, physiological, and epigenetic effects of stress (Barcellos et al., 2016; Barcellos et al., 2011; Koakoski et al., 2014; Nesan and Vijayan, 2016). Stress-related genes are expressed early in life, such as *crf*, proopiomelanocortin (*pomc*), melanocortin 2 receptor (*mc2r*), and steroidogenic acute regulatory protein (*star*) (Oltrabella et al., 2015), while stressor-induced cortisol responses occur after 97 hpf (Alsop and Vijayan, 2009). Thus, the zebrafish represents a useful animal model to investigate the molecular bases underlying human stress physiology (Alderman and Vijayan, 2012; Alsop and Vijayan, 2009; Baiamonte et al., 2015).

Stress represents a response to a stressful condition, such as a threat, challenge, or physical and psychological barrier (Selye, 1976; Ulrich-Lai et al., 2016), while fear and anxiety are primitive emotions to ensure safety when animals respond to challenge (Sylvers et al., 2011). In the clinical literature, fear is a cognitive response to an imminent threat, whereas anxiety is an emotional response to fear (Lang et al., 2000). Thus, failure to extinguish fear responses is a key contributing factor in anxiety- and stress-related disorders (Perathoner et al., 2016; Radulovic et al., 2018). The constructs of fear and anxiety are delineated more clearly in the neuroscience literature. While anxiety is triggered by potentially threatening situations, fear usually occurs in the presence of a real threat. Some stressors usually trigger higher levels of anxiety/fear-like behaviors in zebrafish. For example, both acute conspecific alarm substance (CAS) exposure and net chasing elicit escaping responses and aversive behaviors (Abreu et al., 2014; Barcellos et al., 2011; Cachat et al., 2010; Egan et al., 2009; Mathuru et al., 2012; Mocelin et al., 2015). CAS increases *c-fos* expression in habenula and causes prolonged defensive behaviors, hence characterizing a persistent fear-like response (Maximino et al., 2018; Ogawa et al., 2014; Ogawa et al., 2012). Conversely, net chasing robustly increases cortisol levels, which reflect a high-stress condition (Abreu et al., 2014; Barcellos et al., 2011; Mocelin et al., 2015). Because chemical and mechanical stressors are different in nature, pharmacological interventions aiming to prevent specific stress-induced phenotypes are important.

Taurine (2-aminoethanesulfonic acid) plays a pleiotropic role by modulating osmoregulation (Schaffer et al., 2010), membrane stability (Lambert et al., 2015), intracellular calcium metabolism (Foos and Wu, 2002), and neuronal activity (Wu and Prentice, 2010). Importantly, taurine prevents oxidative stress (Lerdweeraphon et al., 2013) and inflammation (Marcinkiewicz and Kontny, 2014) and hence acts as an endogenous neuroprotector (Menzie et al., 2014) by positively modulating GABA<sub>A</sub> and strychnine-sensitive glycine receptors and inhibiting NMDA receptor activation (Chan et al., 2014; Poleszak et al., 2011; Zhang and Kim, 2007). Because neuroprotective effects of taurine in zebrafish support the existence of different mechanisms of action (Fontana et al., 2016; Fontana et al., 2019; Mezzomo et al., 2016; Rosemberg et al., 2012; Rosemberg et al., 2010), herein, we investigated whether taurine prevents fear/anxiety-like behavioral, neurochemical, and physiological responses in zebrafish submitted to different stressors.

## 2. Materials and methods

### 2.1. Animals

Subjects were adult *short fin* zebrafish (*D. rerio*) (4–6 months old, ~50:50 male-to-female ratio, weighing 0.25–0.4 g) obtained from a

local distributor (Hobby Aquários, Santa Maria, RS). Fish were acclimated in the laboratory for 15 days in 50 L tanks with a maximum density of 2 animals/L; the tanks contained nonchlorinated water kept under constant aeration and mechanical and chemical filtration at  $25 \pm 2$  °C, pH = 7.1. The water conditions were monitored using commercial kits for determining pH, nitrite, and ammonia (Alcon Basic®, Alcon, Brazil). Fluorescent lamp tubes were used to provide illumination by adjusting to a 14/10 h light/dark photoperiod cycle (lights on at 7:00). Fish were fed twice daily with commercial flake food (Alcon Basic®, Alcon, Brazil) and maintained in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. All experimental procedures were approved by the Ethics Committee on Animal Use of the Federal University of Santa Maria (protocol number 106/2014). Moreover, this research was registered in the SisGen (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado) and complied with their guidelines (registration code A14E252).

### 2.2. Experimental design

Our study strategy was to compare behavioral and biochemical stress-related responses in zebrafish in the absence or presence of taurine. The experimental design consisted of exposing fish to water (control group) or taurine (taurine groups) before the induction of acute stress in another tank. Behavioral activities were further analyzed using the novel tank test or the light–dark test. Whole-body cortisol levels and biochemical assays were measured following the behavioral tests.

#### 2.2.1. Taurine pretreatment

To evaluate the effects of taurine (Sigma, St. Louis, MO, USA) on acute stress, we exposed zebrafish individually for 1 h in 500 mL beakers at different taurine concentrations (42, 150, and 400 mg/L). Both exposure period and taurine concentrations were chosen on the basis of those reported in previous studies, which showed positive effects in zebrafish (Fontana et al., 2016; Fontana et al., 2019; Mezzomo et al., 2016; Rosemberg et al., 2012). The water (control) group was kept in nonchlorinated water for the same period. After transferring the fish individually to the behavioral apparatus, the videos were recorded for 6 min and later analyzed using ANY-Maze™ software (Stoelting Co., USA). All behavioral tests were performed between 09:00 and 11:00 AM. Brains were dissected immediately after the behavioral tasks for biochemical assays, and fish submitted to whole-body cortisol extraction were euthanized after 15 min. To ensure data reliability, two independent batches were tested ( $n = 5–7$  per group in each batch).

#### 2.2.2. Acute stress induction

After water or taurine exposure, the induction of stress was performed in another tank using two stressors of different natures. Subsequently, the behavior was analyzed in the novel tank test or in the light–dark test. One cohort of animals was submitted to a mechanical stressor (chasing fish with a net), while another cohort was exposed to a chemical stressor (acute CAS exposure). Mechanical stress was induced by chasing the fish with a net for 2 min as described elsewhere (Abreu et al., 2014; Barcellos et al., 2011; Mocelin et al., 2015). The same trained experimenter executed the net chasing stress protocol (circular clockwise movements with the net, at a regular speed of approximately 40 turns per minute). The protocol of chemical stress induction was performed as described previously (Canzian et al., 2017; Egan et al., 2009; Quadros et al., 2016; Speedie and Gerlai, 2008). CAS was extracted from phenotypically similar donor fish previously euthanized. Briefly, epidermal cells were damaged with 10–15 shallow slices on both sides of the donor fish body with a razor blade. All procedures were performed on ice and carefully controlled to avoid drawing blood, which would contaminate the solution. Afterward, 10 mL of distilled water was added into a Petri dish and gently shaken to fully cover the fish body. Animals were exposed individually to 3.5 mL/L CAS

preparation in 500 mL tanks for 5 min. Control groups were handled in a similar manner as that of the test groups, except that only distilled water was added to the tank.

### 2.3. Behavioral tests

#### 2.3.1. Novel tank diving test

The apparatus was a glass aquarium (20 cm length × 20 cm height × 20 cm width) filled with 1.5 L of home tank water. The apparatus was divided into two equal horizontal areas (bottom and top), and all experimental conditions were similar to those described previously (Egan et al., 2009; Rosemberg et al., 2012; Rosemberg et al., 2011). The following endpoints were measured: time spent in the top area, transitions to the top area, and the number and duration of erratic movements. Erratic movements consist of fast and successive swimming bouts with abrupt changes in direction (Kalueff et al., 2013). Because automated video tracking systems do not precisely quantify some behaviors with the accuracy of human interpretation, erratic movements were manually computed by two observers blinded to the experimental condition (inter-rater reliability > 0.85).

#### 2.3.2. Light–dark test

The light–dark test was performed according to the protocol described previously (Maximino et al., 2010). A rectangular glass tank (25 cm length × 10 cm width × 30 cm height) was divided into two equally sized partitions using a black and white self-adhesive film externally covering the walls, floor, and the corresponding sides of the tank. The apparatus was filled with 2.5 L of home tank water, and after the treatments, fish were removed from the beakers and gently placed in the test apparatus. All behaviors were recorded in a single 6 min session, and the following endpoints were quantified: time spent in the lit area, shuttling, transitions to the lit area, and number of risk assessment episodes. Risk assessment was defined as a fast (> 1 s) entry into the white compartment followed by re-entry into the black compartment, or as a partial entry in the lit area (Kalueff et al., 2013; Maximino et al., 2011). Risk assessment was measured manually by two trained observers blinded to the experimental condition (inter-rater reliability > 0.85).

### 2.4. Biochemical analyses

After behavioral tests, fish were euthanized, and the brains were immediately dissected on ice, transferred to microtubes, and stored at –80 °C. Three brains were pooled per sample and homogenized in 570 µL of 50 mM Tris-HCl buffer, pH 7.4. Samples were further centrifuged (3000 rpm for 10 min, –4 °C), and the supernatants were used for subsequent assays.

### 2.5. Oxidative stress-related parameters

Lipid peroxidation was estimated by thiobarbituric acid reactive substance (TBARS) production as described elsewhere (Draper and Hadley, 1990; Rosemberg et al., 2010). Samples (80 µg protein) were mixed with 10% TCA and further centrifuged at 10,000 × g for 10 min. Supernatants were further mixed with 0.67% thiobarbituric acid and boiled for 30 min. TBARS levels were determined at 532 nm using malondialdehyde (MDA) as standard. Results were expressed as nmol MDA/mg protein. Carbonylated protein (CP) levels were quantified by protein precipitation in the presence of trichloroacetic acid and dinitrophenylhydrazine (DNPH) (Fontana et al., 2019; Yan et al., 1995). Protein samples (200 µL) were mixed with 10 mM DNPH and incubated for 1 h in the dark. Later, 0.15 mL of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing SDS 3.0%), 0.5 mL of heptane (99.5%), and 0.5 mL of ethanol (99.8%) were added sequentially, kept in continuous agitation for 40 s, and centrifuged for 15 min at 1000 × g. The isolated protein was then washed twice by resuspension in ethanol/

ethyl acetate (1:1) and suspended in 0.25 mL of denaturing buffer. CP content was measured spectrophotometrically at 370 nm in a microplate reader and expressed as nanomole carbonyl/milligram protein and calculated using the molar extinction coefficient (22,000 M/cm).

### 2.6. Determination of antioxidant enzymes

Superoxide dismutase (SOD) activity was assessed by measuring the adrenaline oxidation rate at 480 nm as described previously (Misra and Fridovich, 1972). The incubation medium contained glycine–NaOH buffer (50 mM, pH 10), adrenaline (1 mM), and homogenate (20–30 µg of protein). SOD activity was quantified in a microplate reader and expressed as unit SOD/milligram protein (Rosemberg et al., 2010). Catalase (CAT) activity was assessed by measuring the decrease in hydrogen peroxide absorbance at 240 nm by ultraviolet spectrophotometry (Aebi, 1984). The assay mixture had 1 mL potassium phosphate buffer (50 mM, pH 7.0), 0.05 mL H<sub>2</sub>O<sub>2</sub> (0.3 M), and 0.01 mL homogenate (20–30 µg of protein). Results were expressed as U/milligram of protein (Rosemberg et al., 2010).

### 2.7. Protein quantification

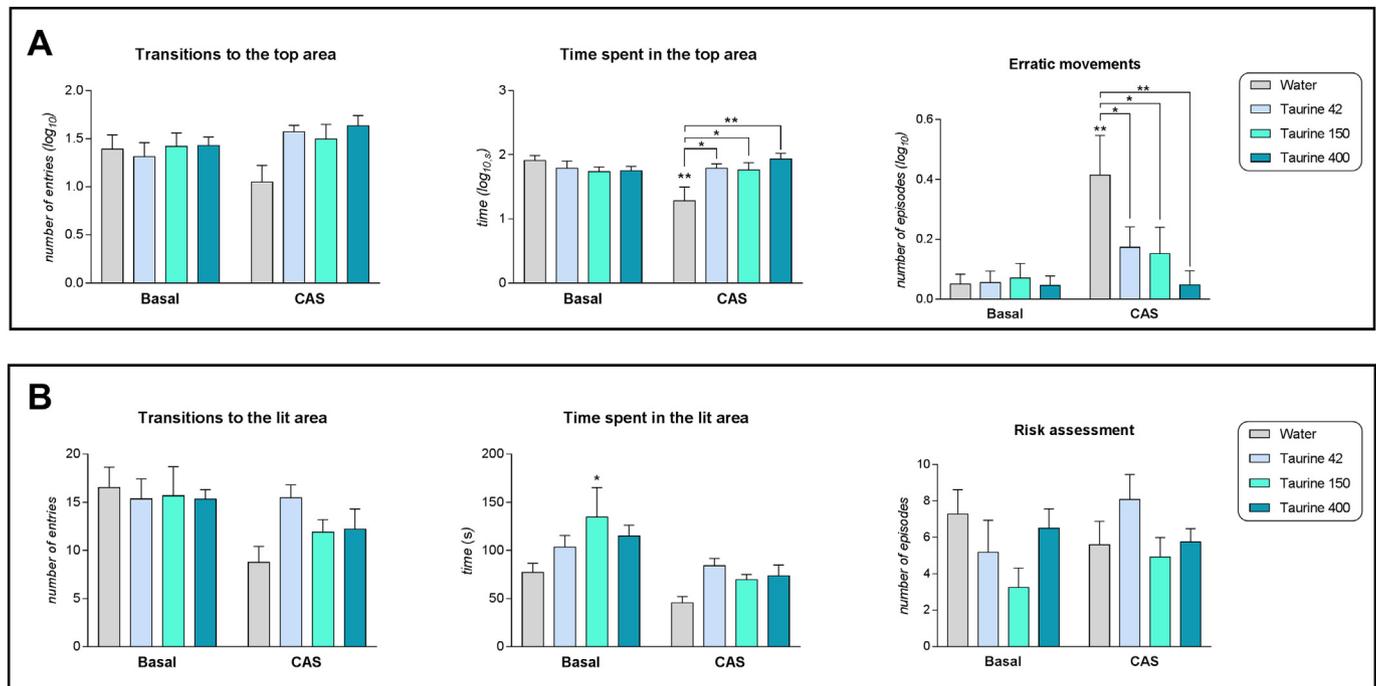
Total protein amount was determined spectrophotometrically by the Coomassie blue method using bovine serum albumin as the standard at 595 nm (Bradford, 1976).

### 2.8. Cortisol extraction and analysis

Whole-body cortisol levels were used as an indicator of stress response. Fish were captured and immediately frozen in liquid nitrogen for 10–30 s, followed by storage at –20 °C until cortisol extraction. Whole-body cortisol was extracted according to the method described elsewhere (Oliveira et al., 2013). Fish were weighed, minced, and placed in a disposable stomacher bag with 2 mL phosphate buffered saline (PBS, pH 7.4) for 6 min. The contents were then transferred to a 10 mL screw top disposable test tube, to which 5 mL of laboratory-grade ethyl ether was added. The tube was vortexed for 1 min and centrifuged for 10 min at 3000 rpm, after which the sample was immediately frozen in liquid nitrogen. The unfrozen portion (ethyl ether containing cortisol) was decanted and transferred to a new tube and completely evaporated under a gentle stream of nitrogen for 2 h, yielding a lipid extract containing cortisol, which was stored at –20 °C. The accuracy was tested by calculating the recoveries from samples spiked with known amounts of cortisol (50, 25, and 12.5 ng/mL). The mean detection of spiked samples was 94.3%. All cortisol values were adjusted for recovery with the following equation: Cortisol value = Measured value × 1.0604. Tissue extracts were resuspended in 1 mL PBS, and whole-body cortisol levels were measured in duplicate for each extraction using a commercially available enzyme-linked immunosorbent assay kit (EIAgen™ Cortisol test, BioChem ImmunoSystems) (Sink et al., 2008). Precision was tested by performing 12 repeated assays on seven randomly chosen samples on the same 96-well plate and calculating the intra-assay coefficient of variation (CV). The reproducibility was assessed by testing the same samples on different plates and calculating the inter-assay CV. To test for linearity and parallelism, tissue samples were subjected to serial dilutions in the buffer provided with the kit. A strong positive correlation between the curves was observed ( $R^2 = 0.8918$ ), and the samples yielded low inter- and intra-assay CV values (7–10% and 5–9%, respectively).

### 2.9. Statistical analysis

Normality of data and homogeneity of variances were analyzed by Kolmogorov–Smirnov and Bartlett's tests, respectively. Nonparametric data were log transformed and analyzed by two-way analysis of variance (ANOVA), considering treatment and stress as factors, followed



**Fig. 1.** Behavioral effects of taurine on CAS-induced chemical stress. (A) Novel tank diving test. (B) Light–dark test. Data were expressed as mean  $\pm$  S.E.M and analyzed by two-way ANOVA, followed by the Student–Newman–Keuls multiple comparison test. Statistical significance was set at  $P \leq 0.05$ . Asterisks above bars express significant differences compared to the control group, while asterisks above brackets indicate statistical differences compared to the CAS group ( $n = 10$ – $14$  animals per group; CAS: conspecific alarm substance; \* $P < 0.05$ , \*\* $P < 0.01$ ).

by Student–Newman–Keuls (SNK) multiple comparison test; results were expressed as mean  $\pm$  standard error of mean (S.E.M). The level of significance was set at  $P \leq 0.05$ , and effect sizes were reported as generalized eta squared ( $\eta^2$ ).

## 3. Results

### 3.1. Behavioral effects

#### 3.1.1. Effects of taurine on CAS-induced chemical stress

Fig. 1A displays the behavioral effects of taurine on CAS-induced chemical stress in the novel tank test. A significant interaction was observed for time spent in the top area [ $F_{3,85} = 5.10$ ,  $P = 0.0027$ ,  $\eta^2 = 0.14$ ], and erratic movements [ $F_{3,85} = 2.74$ ,  $P = 0.0483$ ,  $\eta^2 = 0.32$ ]. Additionally, a CAS effect was observed for number of erratic movements [ $F_{1,85} = 9.58$ ,  $P = 0.0027$ ,  $\eta^2 = 0.09$ ]. CAS-induced chemical stress increased erratic movements and reduced of time spent in the top area, and all treatments abolished these effects.

Fig. 1B shows the behavioral effects of taurine on CAS-induced chemical stress in the light–dark test. Although no interaction effect CAS vs. treatment was observed for transitions to the lit area [ $F_{3,89} = 1.33$ ,  $P = 0.2686$ ,  $\eta^2 = 0.03$ ], or time spent to the lit area [ $F_{3,85} = 1.17$ ,  $P = 0.3265$ ,  $\eta^2 = 0.02$ ], a significant effect of the CAS was observed for time spent to the lit area [ $F_{1,85} = 21.06$ ,  $P = 0.0001$ ,  $\eta^2 = 0.17$ ], and transitions to the lit area [ $F_{1,89} = 6.94$ ,  $P = 0.0099$ ,  $\eta^2 = 0.07$ ]. Meanwhile, a treatment effect was observed for time spent to the lit area [ $F_{3,85} = 4.34$ ,  $P = 0.0068$ ,  $\eta^2 = 0.11$ ], where taurine 150 alone increased time spent to the lit area.

#### 3.1.2. Effects of taurine on chasing-induced mechanical stress

Fig. 2 displays the behavioral effects of taurine on chasing-induced mechanical stress. A significant effect of interaction was observed for time spent in the top area [ $F_{3,96} = 2.94$ ,  $P = 0.0372$ ,  $\eta^2 = 0.08$ ] in the novel tank test (Fig. 2A). No significant effects were observed among treatments in the light–dark test (Fig. 2B).

### 3.2. Biochemical effects

#### 3.2.1. Effects of taurine on CAS-induced chemical stress

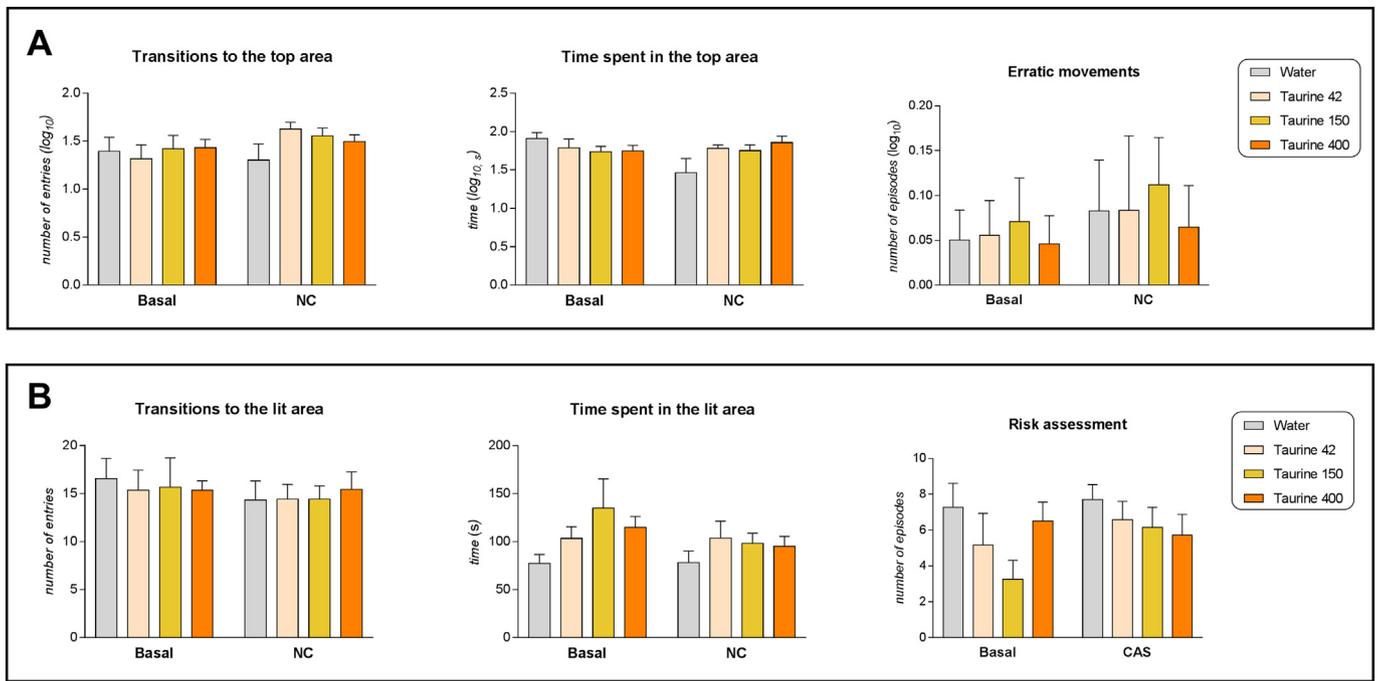
Fig. 3A demonstrates the effects of taurine on CAS-induced chemical stress in oxidative stress-related parameters. A significant interaction was observed for carbonylated proteins levels [ $F_{3,34} = 41.46$ ,  $P < 0.0001$ ,  $\eta^2 = 0.37$ ], and lipid peroxidation [ $F_{3,33} = 4.45$ ,  $P = 0.0098$ ,  $\eta^2 = 0.23$ ]. As well, a CAS effect was detected for carbonylated proteins levels [ $F_{1,34} = 111.5$ ,  $P < 0.0001$ ,  $\eta^2 = 0.33$ ], and lipid peroxidation [ $F_{1,33} = 4.37$ ,  $P = 0.0442$ ,  $\eta^2 = 0.07$ ]. Moreover, a treatment effect was observed for carbonylated proteins levels [ $F_{3,34} = 21.82$ ,  $P < 0.0001$ ,  $\eta^2 = 0.19$ ]. CAS-induced carbonylated proteins levels, and this effect was potentiated in taurine 400 group subjected to stress.

The effects of taurine on antioxidant enzymes are displayed in the Fig. 3B, where an effect of interaction was observed [ $F_{3,33} = 12.78$ ,  $P < 0.0001$ ,  $\eta^2 = 0.39$ ], CAS effect [ $F_{1,33} = 5.23$ ,  $P = 0.0287$ ,  $\eta^2 = 0.05$ ], and treatment [ $F_{3,33} = 7.29$ ,  $P = 0.0007$ ,  $\eta^2 = 0.22$ ] for SOD activity. As a result, SOD activity was markedly potentiated in taurine 400 group subjected to CAS-induced stress. No significant effects were observed in CAT activity.

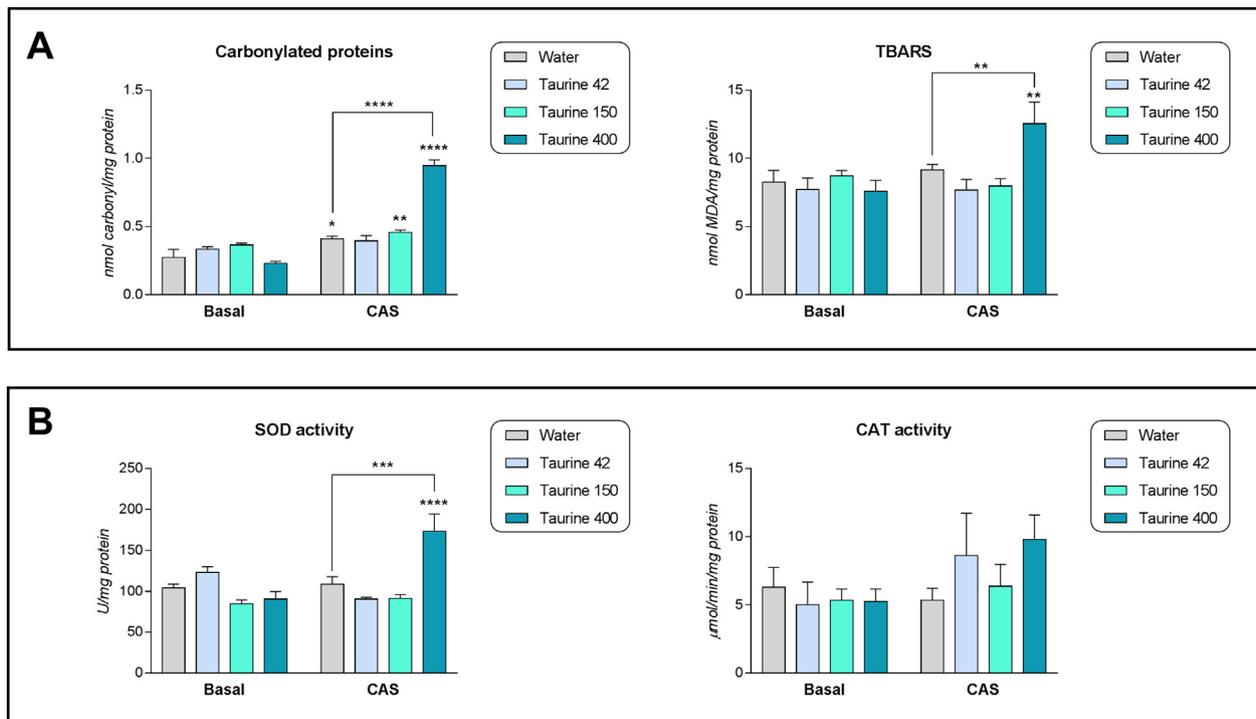
#### 3.2.2. Effects of taurine on chasing-induced mechanical stress

Fig. 4A demonstrates the effects of taurine on chasing-induced mechanical stress in oxidative stress-related parameters. A significant interaction was observed for carbonylated proteins levels [ $F_{3,32} = 34.89$ ,  $P < 0.0001$ ,  $\eta^2 = 0.30$ ]. Additionally, a significant effect of NC was observed for carbonylated proteins levels [ $F_{1,32} = 134.4$ ,  $P < 0.0001$ ,  $\eta^2 = 0.38$ ], and lipid peroxidation [ $F_{1,35} = 110.7$ ,  $P < 0.0001$ ,  $\eta^2 = 0.73$ ]. Furthermore, a significant effect of treatment was observed for carbonylated proteins levels [ $F_{3,32} = 26.86$ ,  $P < 0.0001$ ,  $\eta^2 = 0.23$ ]. Chasing-induced stress induced lipid peroxidation and protein carbonylation. Taurine 42 prevented, and taurine 400 markedly potentiated protein carbonylation.

The effects of taurine on antioxidant enzymes are exhibited in the Fig. 4B. Although no interaction effect NC vs. treatment was revealed



**Fig. 2.** Behavioral effects of taurine on net chasing-induced mechanical stress. (A) Novel tank diving test. (B) Light–dark test. Data were expressed as mean ± S.E.M and analyzed by two-way ANOVA, followed by Student–Newman–Keuls multiple comparison test. Statistical significance was set at  $P \leq 0.05$ . Asterisks above bars express significant differences compared to the control group, while asterisks above brackets indicate statistical differences compared to the NC group ( $n = 10\text{--}14$  animals per group; NC: net chasing).



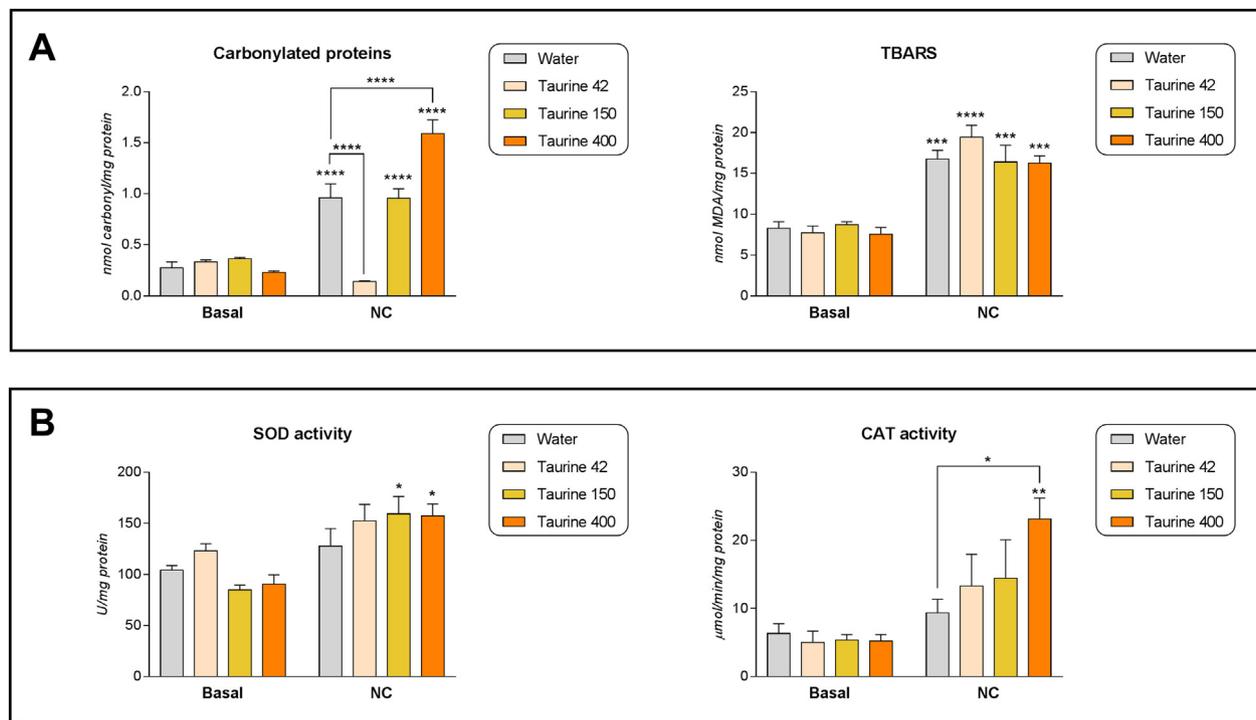
**Fig. 3.** Effects of taurine on CAS-induced chemical stress in biochemical analyses (A) Oxidative stress-related parameters. (B) Antioxidant defenses. Data were expressed as mean ± S.E.M and analyzed by two-way ANOVA, followed by the Student–Newman–Keuls multiple comparison test. Statistical significance was set at  $P \leq 0.05$ . Asterisks above bars express significant differences compared to the control group, while asterisks above brackets indicate statistical differences compared to the CAS group ( $n = 10\text{--}14$  animals per group; CAS: conspecific alarm substance;  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ,  $****P < 0.0001$ ).

for CAT [ $F_{3,31} = 1.96$ ,  $P = 0.1406$ ,  $ges = 0.07$ ], or SOD activities [ $F_{3,33} = 2.10$ ,  $P = 0.1184$ ,  $ges = 0.08$ ], a significant effect of the NC was observed for SOD [ $F_{1,33} = 29.22$ ,  $P < 0.0001$ ,  $ges = 0.40$ ] and CAT activities [ $F_{1,33} = 19.92$ ,  $P < 0.0001$ ,  $ges = 0.32$ ]. SOD activity was increased in taurine 150 and 400 groups, while CAT activity was

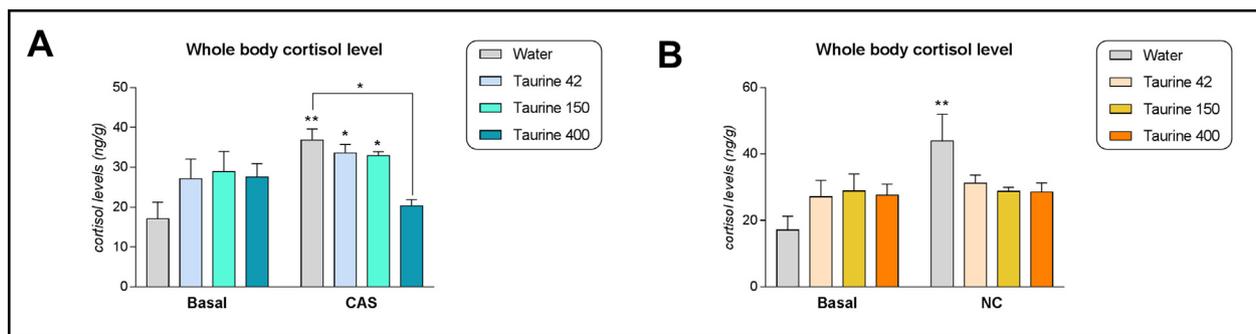
potentiated with taurine 400 group subjected to chasing-induced stress.

### 3.3. Whole-body cortisol

Fig. 5 displays the effects of taurine on whole-body cortisol level.



**Fig. 4.** Effects of taurine on net chasing-induced mechanical stress in biochemical analyses (A) Oxidative stress-related parameters. (B) Antioxidant defenses. Data were expressed as mean  $\pm$  S.E.M and analyzed by two-way ANOVA, followed by Student–Newman–Keuls multiple comparison test. Statistical significance was set at  $P \leq 0.05$ . Asterisks above bars express significant differences compared to the control group, while asterisks above brackets indicate statistical differences compared to the NC group ( $n = 10$ –14 animals per group; NC: net chasing; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ ).



**Fig. 5.** Effects of acute taurine treatment on whole-body cortisol level (A) CAS-induced chemical stress. (B) Net chasing-induced mechanical stress. Data were expressed as mean  $\pm$  S.E.M and analyzed by two-way ANOVA, followed by Student–Newman–Keuls multiple comparison test. Statistical significance was set at  $P \leq 0.05$ . Asterisks above bars express significant differences compared to the control group, while asterisks above brackets indicate statistical differences compared to the CAS/NC group ( $n = 10$ –14 animals per group; CAS: conspecific alarm substance; \* $P < 0.05$ , \*\* $P < 0.01$ ).

The effects of taurine on CAS-induced chemical stress are showed in the Fig. 5A, where an interaction [ $F_{3,33} = 5.12$ ,  $P = 0.0051$ ,  $g_{ES} = 0.26$ ], and CAS [ $F_{1,33} = 5.33$ ,  $P = 0.0273$ ,  $g_{ES} = 0.09$ ] were detected. As a result, a preventive effect of taurine 400 exposed to CAS was observed.

The effects of taurine on chasing-induced mechanical stress showed in the Fig. 5B revealed a significant effect of the interaction ( $F_{3,33} = 42.35$ ,  $P = 0.0123$ ,  $g_{ES} = 0.24$ ) and NC [ $F_{1,33} = 62.51$ ,  $P = 0.0176$ ,  $g_{ES} = 0.12$ ]. No significant effects of the NC or treatment were observed.

#### 4. Discussion

Herein, we show, at least to the best of our knowledge, for the first time a preventive effect of taurine on zebrafish stress responses. In fact, we observed that taurine abolishes some stress-related behaviors (e.g., erratic movements and decrease in vertical exploration). Moreover, depending on the concentration tested, taurine prevents protein

carbonylation and changes in whole-body cortisol levels (taurine 42 and taurine 400, respectively). We suggest that taurine may play a role against some behavioral, neurochemical, and physiological responses triggered by acute stressors in the zebrafish.

To understand stress-related conditions in experimental models, it is necessary to elucidate which stimuli affect defensive behaviors. Although the novel tank test evokes higher levels of cortisol, representing a more stressful procedure than the light–dark test alone (Kysil et al., 2017), both behavioral tasks are used to measure defensive behaviors following acute stress. Here, fish displayed fear- and anxiety-related behaviors after acute stress. More specifically, chemical stress seems to be more effective than mechanical stress to evoke fear-like responses in zebrafish. The net chasing protocol has been described as an effective stressor to fish, which induces a robust increase in whole-body cortisol levels (Abreu et al., 2014; Barcellos et al., 2011; Giacomini et al., 2016; Marcon et al., 2018; Mocelin et al., 2015), corroborating with the data shown here. Differently, CAS increases *c-fos*

expression in habenula (Ogawa et al., 2014) and exacerbates the frequency of erratic movements, suggesting fear (Parra et al., 2009; Speedie and Gerlai, 2008). Although these behaviors are adaptive avoidance responses of a stressful situation (Ferrari et al., 2010), the different contexts described here may serve as important factors in the dynamics of the stress responses that should be further investigated.

Although we did not observe all the anxiolytic-like effects of taurine alone described previously (Mezzomo et al., 2016), only the taurine 150 alone increased time spent to the lit area. Differences in the experimental protocol involving the time interval between the exposure period and the behavioral test could explain these discrepancies. Taurine plays multiple roles in the brain, including neuromodulation and inhibitory neurotransmission (Junyent et al., 2009; Menzie et al., 2014; Mezzomo et al., 2018; Rosemberg et al., 2010). This molecule acts as an agonist of GABA<sub>A</sub> and strychnine-sensitive glycine receptors and can directly interact with the NMDA receptor to suppress its activity (Chan et al., 2014; Poleszak et al., 2011; Zhang and Kim, 2007). Here, taurine prevented anxiety/fear-like behaviors depending on the context of the test. All taurine concentrations chosen abolished CAS-induced changes on erratic movements and geotaxis, which have been considered stress-related phenotypes. Anxiety is a trait typically associated with stressful situations, and the anxiolytic-like effects of taurine in zebrafish were described elsewhere (Mezzomo et al., 2016). Both acute and chronic stresses induce dephosphorylation and down-regulation of the K<sup>+</sup>/Cl<sup>-</sup> co-transporter, which affect the GABAergic control of CRF neurons, which activate physiological response to stress (Corteen et al., 2015; Maguire, 2014; Seifi et al., 2018). Thus, as the effects of taurine in vertebrates may involve GABA<sub>A</sub> activation (Mezzomo et al., 2018), this molecule emerges as a promising alternative strategy for treating stress-related disorders.

Stress disrupts redox homeostasis in the brain, leading to oxidative stress and impairing antioxidant enzyme activities (Dal Santo et al., 2014; Salim, 2017). The involvement of oxidative stress mechanisms has also been suggested in some psychiatric diseases including depression and fear- and anxiety-related disorders (Bouayed et al., 2009; Ng et al., 2008; Salim, 2017; Valko et al., 2007). Acute exposure to stressors increases oxidative stress in zebrafish brain (Dal Santo et al., 2014; Fontana et al., 2019; Maximino et al., 2011; Muller et al., 2018; Muller et al., 2017). The stressors of different natures assessed here stimulated oxidative parameters in all groups. Because taurine has antioxidant properties, this molecule may prevent oxidative stress in the brain (Lerdweeraphon et al., 2013; Rosemberg et al., 2010; Shimada et al., 2015). Here, the lowest concentration of taurine showed protective effects from protein carbonylation, while all other treatments did not prevent oxidative stress-related changes. Importantly, taurine alone did not influence the biochemical parameters measured. CAS- and net chasing-induced stress did not alter the enzymatic antioxidant defenses measured. Nonetheless, pretreatment with the highest taurine concentration stimulated SOD and CAT activities in fish subjected to CAS- and net chasing-induced stress, respectively. Although the lowest taurine concentration showed protective effects against oxidative stress-related changes, as well as stress-related behaviors following CAS exposure, our data do not reflect an associative concentration-dependent effect. Behavioral phenotypes represent complex responses resulting from a multifaceted interaction of various neurotransmitter systems and intricate cell signaling pathways under distinct conditions. Thus, other neurochemical parameters could play a key role in the responses measured here. In general, our results were similar to previous data describing a protective effect of taurine on PTZ-induced oxidative stress in zebrafish (Fontana et al., 2019). Possibly, the modulatory role of taurine on oxidation processes occurs by stimulating enzymatic antioxidant defenses. Vasodilator molecules like taurine increase CAT activity (Furian et al., 2009) and display an important function in controlling oxidative stress-related parameters (Das et al., 2012; Gurer et al., 2001; Parildar-Karpuzoglu et al., 2008). Although more studies are needed to clarify the neurochemical mechanisms underlying the

effects of taurine, our data could reflect a compensatory mechanism that activates antioxidant defenses following a stressful situation.

Similar to humans, cortisol is the main stress hormone in zebrafish following the activation of the HPI axis (Alderman and Vijayan, 2012; Alsop and Vijayan, 2009; Baiamonte et al., 2015). As previously mentioned, stress increases whole-body cortisol content in zebrafish (Fonseka et al., 2016; Giacomini et al., 2016; Oliveira et al., 2013; Tudorache et al., 2013). As expected, both CAS and net chasing protocols increased whole-body cortisol levels in zebrafish. Importantly, pretreatment with the highest taurine concentration dampens the cortisol response to stress. Benzodiazepines modulate the GABA<sub>A</sub> receptor with anxiolytic, hypnotic, and anticonvulsant properties (Gebauer et al., 2011; Low et al., 2000; McKernan et al., 2000). Taurine acts on the GABA<sub>A</sub> receptor, and its potential anxiolytic-like effects are relatively well known in vertebrate models, including zebrafish (Fontana et al., 2019; Mezzomo et al., 2016; Mussulini et al., 2013). Although the exact mechanisms of the biological response of stress are unclear, the use of taurine may serve as an alternative therapeutic tool without benzodiazepine-induced side effects (e.g., sedation).

Usually, psychiatric research is focused on mechanistic explanations underlying fear and anxiety (anxiolytic vs. anxiogenic effects and neurochemical parameters involved). These symptoms naturally support the function to prepare the organism (i.e., physiologically, cognitively, and behaviorally) for detecting and dealing with threats to survival. If some situations may be adaptive to stress, an important question about the consequences of treatment arises. To what extent is the treatment beneficial to dampen the stress response? We suggest the importance of developing new strategies to treat anxiety-, trauma-, and stressor-related disorders.

## 5. Conclusion

In summary, our results show a protective role of taurine against stress-induced behavioral and biochemical changes in zebrafish. Taurine prevents anxiety/fear-like behaviors, protein carbonylation, and cortisol stimulation. Because taurine has beneficial effects in the brain, further studies are necessary to clarify the mechanisms underlying its neuroprotective role in vertebrates.

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## Conflict of interest

The authors declare that no conflict of interest exists.

## References

- Abreu, M.S., Koakoski, G., Ferreira, D., Oliveira, T.A., Rosa, J.G., Gusso, D., Giacomini, A.C., Piato, A.L., Barcellos, L.J., 2014. Diazepam and fluoxetine decrease the stress response in zebrafish. *PLoS One* 9, e103232.
- Aebi, H., 1984. Catalase in vitro. *Methods Enzymol.* 105, 121–126.
- Agetsuma, M., Aizawa, H., Aoki, T., Nakayama, R., Takahoko, M., Goto, M., Sassa, T., Amo, R., Shiraki, T., Kawakami, K., Hosoya, T., Higashijima, S., Okamoto, H., 2010.

- The habenula is crucial for experience-dependent modification of fear responses in zebrafish. *Nat. Neurosci.* 13, 1354–1356.
- Agostini, J.F., Toe, H., Vieira, K.M., Baldin, S.L., Costa, N.L.F., Cruz, C.U., Longo, L., Machado, M.M., da Silveira, T.R., Schuck, P.F., Rico, E.P., 2018. Cholinergic system and oxidative stress changes in the brain of a zebrafish model chronically exposed to ethanol. *Neurotox. Res.* 33, 749–758.
- Alderman, S.L., Vijayan, M.M., 2012. 11 $\beta$ -Hydroxysteroid dehydrogenase type 2 in zebrafish brain: a functional role in hypothalamus-pituitary-interrenal axis regulation. *J. Endocrinol.* 215, 393–402.
- Alsop, D., Vijayan, M., 2009. The zebrafish stress axis: molecular fallout from the teleost-specific genome duplication event. *Gen. Comp. Endocrinol.* 161, 62–66.
- Baiamonte, M., Brennan, C.H., Vinson, G.P., 2015. Sustained action of developmental ethanol exposure on the cortisol response to stress in zebrafish larvae and adults. *PLoS One* 10, e0124488.
- Barcellos, L.J., Volpato, G.L., Barreto, R.E., Coldebella, I., Ferreira, D., 2011. Chemical communication of handling stress in fish. *Physiol. Behav.* 103, 372–375.
- Barcellos, H.H., Kalichak, F., da Rosa, J.G., Oliveira, T.A., Koakoski, G., Idalencio, R., de Abreu, M.S., Giacomini, A.C., Fagundes, M., Variani, C., Rossini, M., Piato, A.L., Barcellos, L.J., 2016. Waterborne aripiprazole blunts the stress response in zebrafish. *Sci. Rep.* 6, 37612.
- Bouayed, J., Rammal, H., Soulimani, R., 2009. Oxidative stress and anxiety: relationship and cellular pathways. *Oxidative Med. Cell. Longev.* 2, 63–67.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Brennan, C.H., 2011. Zebrafish behavioural assays of translational relevance for the study of psychiatric disease. *Rev. Neurosci.* 22, 37–48.
- Bury, N.R., Sturm, A., 2007. Evolution of the corticosteroid receptor signalling pathway in fish. *Gen. Comp. Endocrinol.* 153, 47–56.
- Cachat, J., Stewart, A., Grossman, L., Gaikwad, S., Kadri, F., Chung, K.M., Wu, N., Wong, K., Roy, S., Suci, C., Goodspeed, J., Elegante, M., Bartels, B., Elkhatay, S., Tien, D., Tan, J., Denmark, A., Gilder, T., Kyzar, E., Dileo, J., Frank, K., Chang, K., Utterback, E., Hart, P., Kalueff, A.V., 2010. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. *Nat. Protoc.* 5, 1786–1799.
- Canzian, J., Fontana, B.D., Quadros, V.A., Rosemberg, D.B., 2017. Conspecific alarm substance differently alters group behavior of zebrafish populations: putative involvement of cholinergic and purinergic signaling in anxiety- and fear-like responses. *Behav. Brain Res.* 320, 255–263.
- Chan, C.Y., Sun, H.S., Shah, S.M., Agovic, M.S., Friedman, E., Banerjee, S.P., 2014. Modes of direct modulation by taurine of the glutamate NMDA receptor in rat cortex. *Eur. J. Pharmacol.* 728, 167–175.
- Corteen, N.L., Carter, J.A., Rudolph, U., Belleli, D., Lambert, J.J., Swinny, J.D., 2015. Localisation and stress-induced plasticity of GABAA receptor subunits within the cellular networks of the mouse dorsal raphe nucleus. *Brain Struct. Funct.* 220, 2739–2763.
- Dal Santo, G., Conterato, G.M., Barcellos, L.J., Rosemberg, D.B., Piato, A.L., 2014. Acute restraint stress induces an imbalance in the oxidative status of the zebrafish brain. *Neurosci. Lett.* 558, 103–108.
- Das, J., Vasan, V., Sil, P.C., 2012. Taurine exerts hypoglycemic effect in alloxan-induced diabetic rats, improves insulin-mediated glucose transport signaling pathway in heart and ameliorates cardiac oxidative stress and apoptosis. *Toxicol. Appl. Pharmacol.* 258, 296–308.
- Draper, H.H., Hadley, M., 1990. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.* 186, 421–431.
- Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhatay, 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.
- Ferrari, M.C., Brown, G.E., Bortolotti, G.R., Chivers, D.P., 2010. Linking predator risk and uncertainty to adaptive forgetting: a theoretical framework and empirical test using tadpoles. *Proc. Biol. Sci.* 277, 2205–2210.
- Fonseka, T.M., Wen, X.Y., Foster, J.A., Kennedy, S.H., 2016. Zebrafish models of major depressive disorders. *J. Neurosci. Res.* 94, 3–14.
- Fontana, B.D., Meinerz, D.L., Rosa, L.V., Mezzomo, N.J., Silveira, A., Giuliani, G.S., Quadros, V.A., Filho, G.L., Blaser, R.E., Rosemberg, D.B., 2016. Modulatory action of taurine on ethanol-induced aggressive behavior in zebrafish. *Pharmacol. Biochem. Behav.* 141, 18–27.
- Fontana, B.D., Ziani, P.R., Canzian, J., Mezzomo, N.J., Muller, T.E., Dos Santos, M.M., Loro, V.L., Barbosa, N.V., Mello, C.F., Rosemberg, D.B., 2019. Taurine protects from pentylentetrazole-induced behavioral and neurochemical changes in zebrafish. *Mol. Neurobiol.* 56 (1), 583–594.
- Foos, T.M., Wu, J.Y., 2002. The role of taurine in the central nervous system and the modulation of intracellular calcium homeostasis. *Neurochem. Res.* 27, 21–26.
- Furian, A.F., Rattmann, Y.D., Oliveira, M.S., Royes, L.F., Marques, M.C., Santos, A.R., Mello, C.F., 2009. Nitric oxide and potassium channels mediate GM1 ganglioside-induced vasorelaxation. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 380, 487–495.
- Fuzzen, M.L., Van Der Kraak, G., Bernier, N.J., 2010. Stirring up new ideas about the regulation of the hypothalamic-pituitary-interrenal axis in zebrafish (*Danio rerio*). *Zebrafish* 7, 349–358.
- Gebauer, D.L., Pagnussat, N., Piato, A.L., Schaefer, I.C., Bonan, C.D., Lara, D.R., 2011. Effects of anxiolytics in zebrafish: similarities and differences between benzodiazepines, buspirone and ethanol. *Pharmacol. Biochem. Behav.* 99, 480–486.
- Gerlai, R., 2010. Zebrafish antidepressant responses: a future for translational research? *Behav. Brain Res.* 207, 223–231.
- Ghisleni, G., Capiotti, K.M., Da Silva, R.S., Oses, J.P., Piato, A.L., Soares, V., Bogo, M.R., Bonan, C.D., 2012. The role of CRH in behavioral responses to acute restraint stress in zebrafish. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 36, 176–182.
- Giacomini, A.C., Abreu, M.S., Zandrea, R., Saibt, N., Friedrich, M.T., Koakoski, G., Gusso, D., Piato, A.L., Barcellos, L.J., 2016. Environmental and pharmacological manipulations blunt the stress response of zebrafish in a similar manner. *Sci. Rep.* 6, 28986.
- Gurer, H., Ozgunes, H., Saygin, E., Ercal, N., 2001. Antioxidant effect of taurine against lead-induced oxidative stress. *Arch. Environ. Contam. Toxicol.* 41, 397–402.
- Holsboer, F., 2000. The stress hormone system is back on the map. *Curr. Psychiatry Rep.* 2, 454–456.
- Junyent, F., Utrera, J., Romero, R., Pallas, M., Camins, A., Duque, D., Auladell, C., 2009. Prevention of epilepsy by taurine treatments in mice experimental model. *J. Neurosci. Res.* 87, 1500–1508.
- Kalueff, A.V., Gebhardt, M., Stewart, A.M., Cachat, J.M., Brimmer, M., Chawla, J.S., Craddock, C., Kyzar, E.J., Roth, A., Landsman, S., Gaikwad, S., Robinson, K., Baatrup, E., Tierney, K., Shamchuk, A., Norton, W., Miller, N., Nicolson, T., Braubach, O., Gilman, C.P., Pittman, J., Rosemberg, D.B., Gerlai, R., Echevarria, D., Lamb, E., Neuhaus, S.C., Weng, W., Bally-Cuif, L., Schneider, H., Zebrafish Neuroscience Research, C., 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. *Zebrafish* 10, 70–86.
- Kalueff, A.V., Stewart, A.M., Gerlai, R., 2014. Zebrafish as an emerging model for studying complex brain disorders. *Trends Pharmacol. Sci.* 35, 63–75.
- Kastenhuber, E., Kratochwil, C.F., Ryu, S., Schweitzer, J., Driever, W., 2010. Genetic dissection of dopaminergic and noradrenergic contributions to catecholaminergic tracts in early larval zebrafish. *J. Comp. Neurol.* 518, 439–458.
- Koakoski, G., Quevedo, R.M., Ferreira, D., Oliveira, T.A., da Rosa, J.G., de Abreu, M.S., Gusso, D., Marqueze, A., Kreutz, L.C., Giacomini, A.C., Fagundes, M., Barcellos, L.J., 2014. Agrichemicals chronically inhibit the cortisol response to stress in fish. *Chemosphere* 112, 85–91.
- Kysil, E.V., Meshalkina, D.A., Frick, E.E., Echevarria, D.J., Rosemberg, D.B., Maximino, C., Lima, M.G., Abreu, M.S., Giacomini, A.C., Barcellos, L.J.G., Song, C., Kalueff, A.V., 2017. Comparative analyses of zebrafish anxiety-like behavior using conflict-based novelty tests. *Zebrafish* 14, 197–208.
- Lambert, I.H., Kristensen, D.M., Holm, J.B., Mortensen, O.H., 2015. Physiological role of taurine—from organism to organelle. *Acta Physiol (Oxford)* 213, 191–212.
- Lang, P.J., Davis, M., Ohman, A., 2000. Fear and anxiety: animal models and human cognitive psychophysiology. *J. Affect. Disord.* 61, 137–159.
- Lerdweeraphon, W., Wyss, J.M., Boomars, T., Roysommut, S., 2013. Perinatal taurine exposure affects adult oxidative stress. *Am. J. Phys. Regul. Integr. Comp. Phys.* 305, R95–R97.
- Lillesaar, C., Tannhauser, B., Stigloher, C., Kremmer, E., Bally-Cuif, L., 2007. The serotonergic phenotype is acquired by converging genetic mechanisms within the zebrafish central nervous system. *Dev. Dyn.* 236, 1072–1084.
- Low, K., Crestani, F., Keist, R., Benke, D., Brunig, I., Benson, J.A., Fritschy, J.M., Rulicke, T., Bluethmann, H., Mohler, H., Rudolph, U., 2000. Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* 290, 131–134.
- Maguire, J., 2014. Stress-induced plasticity of GABAergic inhibition. *Front. Cell. Neurosci.* 8, 157.
- Marcinkiewicz, J., Kontny, E., 2014. Taurine and inflammatory diseases. *Amino Acids* 46, 7–20.
- Marcon, M., Mocelin, R., Benvenuti, R., Costa, T., Herrmann, A.P., de Oliveira, D.L., Koakoski, G., Barcellos, L.J.G., Piato, A., 2018. Environmental enrichment modulates the response to chronic stress in zebrafish. *J. Exp. Biol.* 221.
- Mathuru, A.S., Jesuthasan, S., 2013. The medial habenula as a regulator of anxiety in adult zebrafish. *Front. Neural. Circuit.* 7, 99.
- Mathuru, A.S., Kibat, C., Cheong, W.F., Shui, G., Wenk, M.R., Friedrich, R.W., Jesuthasan, S., 2012. Chondroitin fragments are odorants that trigger fear behavior in fish. *Curr. Biol.* 22, 538–544.
- Maximino, C., de Brito, T.M., da Silva Batista, A.W., Herculano, A.M., Morato, S., Gouveia Jr., A., 2010. Measuring anxiety in zebrafish: a critical review. *Behav. Brain Res.* 214, 157–171.
- Maximino, C., da Silva, A.W., Gouveia Jr., A., Herculano, A.M., 2011. Pharmacological analysis of zebrafish (*Danio rerio*) scototaxis. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 35, 624–631.
- Maximino, C., Meinerz, D.L., Fontana, B.D., Mezzomo, N.J., Stefanello, F.V., de S. Prestes, A., Batista, C.B., Rubin, M.A., Barbosa, N.V., Rocha, J.B.T., Lima, M.G., Rosemberg, D.B., 2018. Extending the analysis of zebrafish behavioral endophenotypes for modeling psychiatric disorders: fear conditioning to conspecific alarm response. *Behav. Processes.* 149, 35–42.
- McKernan, R.M., Rosahl, T.W., Reynolds, D.S., Sur, C., Wafford, K.A., Atack, J.R., Farrar, S., Myers, J., Cook, G., Ferris, P., Garrett, L., Bristow, L., Marshall, G., Macaulay, A., Brown, N., Howell, O., Moore, K.W., Carling, R.W., Street, L.J., Castro, J.L., Ragan, C.I., Dawson, G.R., Whiting, P.J., 2000. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor  $\alpha 1$  subtype. *Nat. Neurosci.* 3, 587–592.
- Menzie, J., Pan, C., Prentice, H., Wu, J.Y., 2014. Taurine and central nervous system disorders. *Amino Acids* 46, 31–46.
- Mezzomo, N.J., Silveira, A., Giuliani, G.S., Quadros, V.A., Rosemberg, D.B., 2016. The role of taurine on anxiety-like behaviors in zebrafish: a comparative study using the novel tank and the light-dark tasks. *Neurosci. Lett.* 613, 19–24.
- Mezzomo, N.J., Fontana, B.D., Kalueff, A.V., Barcellos, L.J.G., Rosemberg, D.B., 2018. Understanding taurine CNS activity using alternative zebrafish models. *Neurosci. Biobehav. Rev.* 90, 471–485.
- Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* 247, 3170–3175.

- Mocelin, R., Herrmann, A.P., Marcon, M., Rambo, C.L., Rohden, A., Bevilacqua, F., de Abreu, M.S., Zanatta, L., Elisabetsky, E., Barcellos, L.J., Lara, D.R., Piato, A.L., 2015. N-acetylcysteine prevents stress-induced anxiety behavior in zebrafish. *Pharmacol. Biochem. Behav.* 139 (Pt B), 121–126.
- Moreno-Peral, P., Conejo-Ceron, S., Motrico, E., Rodriguez-Morejon, A., Fernandez, A., Garcia-Campayo, J., Roca, M., Serrano-Blanco, A., Rubio-Valera, M., Bellon, J.A., 2014. Risk factors for the onset of panic and generalised anxiety disorders in the general adult population: a systematic review of cohort studies. *J. Affect. Disord.* 168, 337–348.
- Muller, T.E., Nunes, S.Z., Silveira, A., Loro, V.L., Rosemberg, D.B., 2017. Repeated ethanol exposure alters social behavior and oxidative stress parameters of zebrafish. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 79, 105–111.
- Muller, T.E., Nunes, M.E., Menezes, C.C., Marins, A.T., Leitemperger, J., Gressler, A.C.L., Carvalho, F.B., de Freitas, C.M., Quadros, V.A., Fachinnetto, R., Rosemberg, D.B., Loro, V.L., 2018. Sodium selenite prevents paraquat-induced neurotoxicity in zebrafish. *Mol. Neurobiol.* 55, 1928–1941.
- Mussulini, B.H., Leite, C.E., Zenki, K.C., Moro, L., Baggio, S., Rico, E.P., Rosemberg, D.B., Dias, R.D., Souza, T.M., Calcagnotto, M.E., Campos, M.M., Battastini, A.M., de Oliveira, D.L., 2013. Seizures induced by pentylenetetrazole in the adult zebrafish: a detailed behavioral characterization. *PLoS One* 8, e54515.
- Nesan, D., Vijayan, M.M., 2016. Maternal cortisol mediates hypothalamus-pituitary-interrunal axis development in zebrafish. *Sci. Rep.* 6, 22582.
- Newport, D.J., Nemeroff, C.B., 2000. Neurobiology of posttraumatic stress disorder. *Curr. Opin. Neurobiol.* 10, 211–218.
- Ng, F., Berk, M., Dean, O., Bush, A.I., 2008. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *Int. J. Neuropsychopharmacol.* 11, 851–876.
- Norton, W.H., 2013. Toward developmental models of psychiatric disorders in zebrafish. *Front. Neural. Circuit.* 7, 79.
- Norton, W., Bally-Cuif, L., 2010. Adult zebrafish as a model organism for behavioural genetics. *BMC Neurosci.* 11, 90.
- Ogawa, S., Ng, K.W., Ramadasan, P.N., Nathan, F.M., Parhar, I.S., 2012. Habenular Kiss1 neurons modulate the serotonergic system in the brain of zebrafish. *Endocrinology* 153, 2398–2407.
- Ogawa, S., Nathan, F.M., Parhar, I.S., 2014. Habenular kisspeptin modulates fear in the zebrafish. *Proc. Natl. Acad. Sci. U. S. A.* 111, 3841–3846.
- Oliveira, R.F., 2013. Mind the fish: zebrafish as a model in cognitive social neuroscience. *Front. Neural. Circuit.* 7, 131.
- Oliveira, T.A., Koakoski, G., Kreutz, L.C., Ferreira, D., da Rosa, J.G., de Abreu, M.S., Giacomini, A.C., Oliveira, R.P., Fagundes, M., Piato, A.L., Barreto, R.E., Barcellos, L.J., 2013. Alcohol impairs predation risk response and communication in zebrafish. *PLoS One* 8, e75780.
- Oltrabella, F., Pietka, G., Ramirez, I.B., Mironov, A., Starborg, T., Drummond, I.A., Hinchliffe, K.A., Lowe, M., 2015. The Lowe syndrome protein OCLL1 is required for endocytosis in the zebrafish pronephric tubule. *PLoS Genet.* 11, e1005058.
- Parildar-Karpuzoglu, H., Mehmetcik, G., Ozdemirler-Erata, G., Dogru-Abbasoglu, S., Kocak-Toker, N., Uysal, M., 2008. Effect of taurine treatment on pro-oxidant-antioxidant balance in livers and brains of old rats. *Pharmacol. Rep.* 60, 673–678.
- Parker, M.O., Millington, M.E., Combe, F.J., Brennan, C.H., 2012. Housing conditions differentially affect physiological and behavioural stress responses of zebrafish, as well as the response to anxiolytics. *PLoS One* 7, e34992.
- Parra, K.V., Adrian Jr., J.C., Gerlai, R., 2009. The synthetic substance hypoxanthine 3-N-oxide elicits alarm reactions in zebrafish (*Danio rerio*). *Behav. Brain Res.* 205, 336–341.
- Perathoner, S., Cordero-Maldonado, M.L., Crawford, A.D., 2016. Potential of zebrafish as a model for exploring the role of the amygdala in emotional memory and motivational behavior. *J. Neurosci. Res.* 94, 445–462.
- Poleszak, E., Socala, K., Szopa, A., Wrobel, A., Szewczyk, B., Kasperek, R., Blicharska, E., Nowak, G., Wlaz, P., 2011. Involvement of NMDA receptor complex in the anxiolytic-like effects of chlordiazepoxide in mice. *J. Neural Transm. (Vienna)* 118, 857–864.
- Quadros, V.A., Silveira, A., Giuliani, G.S., Didonet, F., Silveira, A.S., Nunes, M.E., Silva, T.O., Loro, V.L., Rosemberg, D.B., 2016. Strain- and context-dependent behavioural responses of acute alarm substance exposure in zebrafish. *Behav. Process.* 122, 1–11.
- Radulovic, J., Ren, L.Y., Gao, C., 2018. N-methyl D-aspartate receptor subunit signaling in fear extinction. *Psychopharmacology* (in press).
- Randlett, O., Wee, C.L., Naumann, E.A., Nnaemeka, O., Schoppik, D., Fitzgerald, J.E., Portugues, R., Lacoste, A.M., Riegler, C., Engert, F., Schier, A.F., 2015. Whole-brain activity mapping onto a zebrafish brain atlas. *Nat. Methods* 12, 1039–1046.
- Rosemberg, D.B., da Rocha, R.F., Rico, E.P., Zanotto-Filho, A., Dias, R.D., Bogo, M.R., Bonan, C.D., Moreira, J.C., Klamt, F., Souza, D.O., 2010. Taurine prevents enhancement of acetylcholinesterase activity induced by acute ethanol exposure and decreases the level of markers of oxidative stress in zebrafish brain. *Neuroscience* 171, 683–692.
- Rosemberg, D.B., Rico, E.P., Mussulini, B.H., Piato, A.L., Calcagnotto, M.E., Bonan, C.D., Dias, R.D., Blaser, R.E., Souza, D.O., de Oliveira, D.L., 2011. Differences in spatio-temporal behavior of zebrafish in the open tank paradigm after a short-period confinement into dark and bright environments. *PLoS One* 6, e19397.
- Rosemberg, D.B., Braga, M.M., Rico, E.P., Loss, C.M., Cordova, S.D., Mussulini, B.H., Blaser, R.E., Leite, C.E., Campos, M.M., Dias, R.D., Calcagnotto, M.E., de Oliveira, D.L., Souza, D.O., 2012. Behavioral effects of taurine pretreatment in zebrafish acutely exposed to ethanol. *Neuropharmacology* 63, 613–623.
- Salim, S., 2017. Oxidative stress and the central nervous system. *J. Pharmacol. Exp. Ther.* 360, 201–205.
- Schaffer, S.W., Jong, C.J., Ramila, K.C., Azuma, J., 2010. Physiological roles of taurine in heart and muscle. *J. Biomed. Sci.* 17 (Suppl. 1), S2.
- Schweitzer, J., Driever, W., 2009. Development of the dopamine systems in zebrafish. *Adv. Exp. Med. Biol.* 651, 1–14.
- Seifi, M., Rodaway, S., Rudolph, U., Swinny, J.D., 2018. GABAA receptor subtypes regulate stress-induced colon inflammation in mice. *Gastroenterology* 155 (3), 852–864.e3.
- Selye, H., 1976. The stress concept. *Can. Med. Assoc. J.* 115, 718.
- Shimada, K., Jong, C.J., Takahashi, K., Schaffer, S.W., 2015. Role of ROS production and turnover in the antioxidant activity of taurine. *Adv. Exp. Med. Biol.* 803, 581–596.
- Sink, T.D., Lochmann, R.T., Fecteau, K.A., 2008. Validation, use, and disadvantages of enzyme-linked immunosorbent assay kits for detection of cortisol in channel catfish, largemouth bass, red pacu, and golden shiners. *Fish Physiol. Biochem.* 34, 95–101.
- Sison, M., Cawker, J., Buske, C., Gerlai, R., 2006. Fishing for genes influencing vertebrate behavior: zebrafish making headway. *Lab Anim.* 35, 33–39.
- Speedie, N., Gerlai, R., 2008. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). *Behav. Brain Res.* 188, 168–177.
- Steenbergen, P.J., Richardson, M.K., Champagne, D.L., 2011. The use of the zebrafish model in stress research. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 35, 1432–1451.
- Sylvers, P., Lilienfeld, S.O., LaPrairie, J.L., 2011. Differences between trait fear and trait anxiety: implications for psychopathology. *Clin. Psychol. Rev.* 31, 122–137.
- Tran, S., Chatterjee, D., Gerlai, R., 2014. Acute net stressor increases whole-body cortisol levels without altering whole-brain monoamines in zebrafish. *Behav. Neurosci.* 128, 621–624.
- Tudorache, C., Schaaf, M.J., Slabbekoorn, H., 2013. Covariation between behaviour and physiology indicators of coping style in zebrafish (*Danio rerio*). *J. Endocrinol.* 219, 251–258.
- Ullmann, J.F., Cowin, G., Kurniawan, N.D., Collin, S.P., 2010. A three-dimensional digital atlas of the zebrafish brain. *NeuroImage* 51, 76–82.
- Ulrich-Lai, Y.M., Christiansen, A.M., Wang, X., Song, S., Herman, J.P., 2016. Statistical modeling implicates neuroanatomical circuit mediating stress relief by 'comfort' food. *Brain Struct. Funct.* 221, 3141–3156.
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* 39, 44–84.
- Vijayan, M.M., Walsh, P.J., Mommmsen, T.P., 2016. Endocrine control of metabolism: a tribute to Professor T.W. Moon on the occasion of his retirement. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 199, 1–3.
- Walker, E.F., Trotman, H.D., Pearce, B.D., Addington, J., Cadenhead, K.S., Cornblatt, B.A., Heinsen, R., Mathalon, D.H., Perkins, D.O., Seidman, L.J., Tsuang, M.T., Cannon, T.D., McGlashan, T.H., Woods, S.W., 2013. Cortisol levels and risk for psychosis: initial findings from the North American prodrome longitudinal study. *Biol. Psychiatry* 74, 410–417.
- Wu, J.Y., Prentice, H., 2010. Role of taurine in the central nervous system. *J. Biomed. Sci.* 17 (Suppl. 1), S1.
- Yan, L.J., Droy-Lefaix, M.T., Packer, L., 1995. Ginkgo biloba extract (EGb 761) protects human low density lipoproteins against oxidative modification mediated by copper. *Biochem. Biophys. Res. Commun.* 212, 360–366.
- Zhang, C.G., Kim, S.J., 2007. Taurine induces anti-anxiety by activating strychnine-sensitive glycine receptor in vivo. *Ann. Nutr. Metab.* 51, 379–386.