Journal of Psychiatric Research 119 (2019) 14–22

Contents lists available at ScienceDirect

Journal of Psychiatric Research

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

Modeling psychiatric comorbid symptoms of epileptic seizures in zebrafish

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ARTICLE INFO

Keywords:
Epilepsy-related comorbidities
Social behavior
Exploratory activity
Anxiety
Memory deficits
Zebrafish

ABSTRACT

Epilepsy is a debilitating neurological disorder characterized by recurrent unprovoked seizures. Anxiety, cognitive deficits, depressive-like symptoms, and social dysfunction are psychiatric comorbidities with high prevalence in epileptic patients. Due to the genetic and behavioral tractability, the zebrafish is a promising model organism to understand the neural bases involved in epilepsy-related comorbidities. Here, we aimed to characterize some behavioral phenotypes paralleling those observed in epilepsy-related comorbidities after a single pentylenetetrazole (PTZ) exposure in zebrafish. We also analyzed the influence of whole-body cortisol levels in the behavioral responses measured. Fish were exposed to 10 mM PTZ for 20 min to induce epileptic seizures. After 24 h recovery period, locomotion and anxiety-like responses (novel tank and light-dark tests), social interaction (shoaling behavior task), and memory retention (inhibitory avoidance protocol) were assessed. Basically, PTZ impaired habituation to novelty stress, evoked anxiogenic-like behaviors, disrupted shoaling, and caused memory consolidation deficits in zebrafish without changing whole-body cortisol levels. In conclusion, our novel findings further validate the use of zebrafish as a suitable tool for modeling epilepsy-related comorbidities in translational neuropsychiatric research.

1. Introduction

Epilepsy is a neurological disorder characterized by recurrent epileptic seizures and unpredictable disruptions of brain homeostasis (Sierra-Paredes and Sierra-Marcuno, 2007). This debilitating condition affects 1–2% of the population (~70 million people worldwide) (Sierra-Paredes and Sierra-Marcuno, 2007; Singh and Trevick, 2016). Because epileptic seizures have a multifactorial basis (Engel et al., 2013), the mechanisms underlying the neurobehavioral responses observed in the recovery period are still obscure. Basically, epilepsy involves increased glutamatergic excitability and decreased GABAergic function in the central nervous system (CNS) (Sierra-Paredes and Sierra-Marcuno, 2007). The exposure to chemical agents, such as pentylenetetrazole (PTZ), which antagonizes $GABA_A$ receptors in the brain (Huang et al., 2001), is commonly used to measure seizure-like phenotypes in experimental models (Dhir, 2012; Mussulini et al., 2013). Furthermore, epileptic seizures may trigger various comorbidities in humans and rodents, including anxiety, cognitive deficits, depressive-like symptoms, and social dysfunction (Di Liberto et al., 2018; Henbid et al., 2017; Hunter et al., 2019; Zhu et al., 2018).

Mounting evidence demonstrates a correlation between epilepsy and psychiatric comorbidities. For example, depression- and anxiety-related disorders are described in 10–30% pediatric and adult epileptic patients (Tellez-Zenteno et al., 2007). Treatments for anxiety-related disorders that modulate glutamatergic activity also evoke anxiolysis (Murrough et al., 2015), suggesting that psychiatric comorbidities and epilepsy may share similar underlying mechanisms (Mazarati, 2016). Because seizures and psychiatric comorbidities are associated with changes in the CNS homeostasis, the validation of alternative model organisms in psychiatric research is needed.

The zebrafish (*Danio rerio*) is emerging as an interesting tool in neuropsychiatric research (Fontana et al., 2018). This species displays

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https://doi.org/10.1016/j.jpsychires.2019.09.007
Received 10 May 2019; Received in revised form 31 July 2019; Accepted 12 September 2019

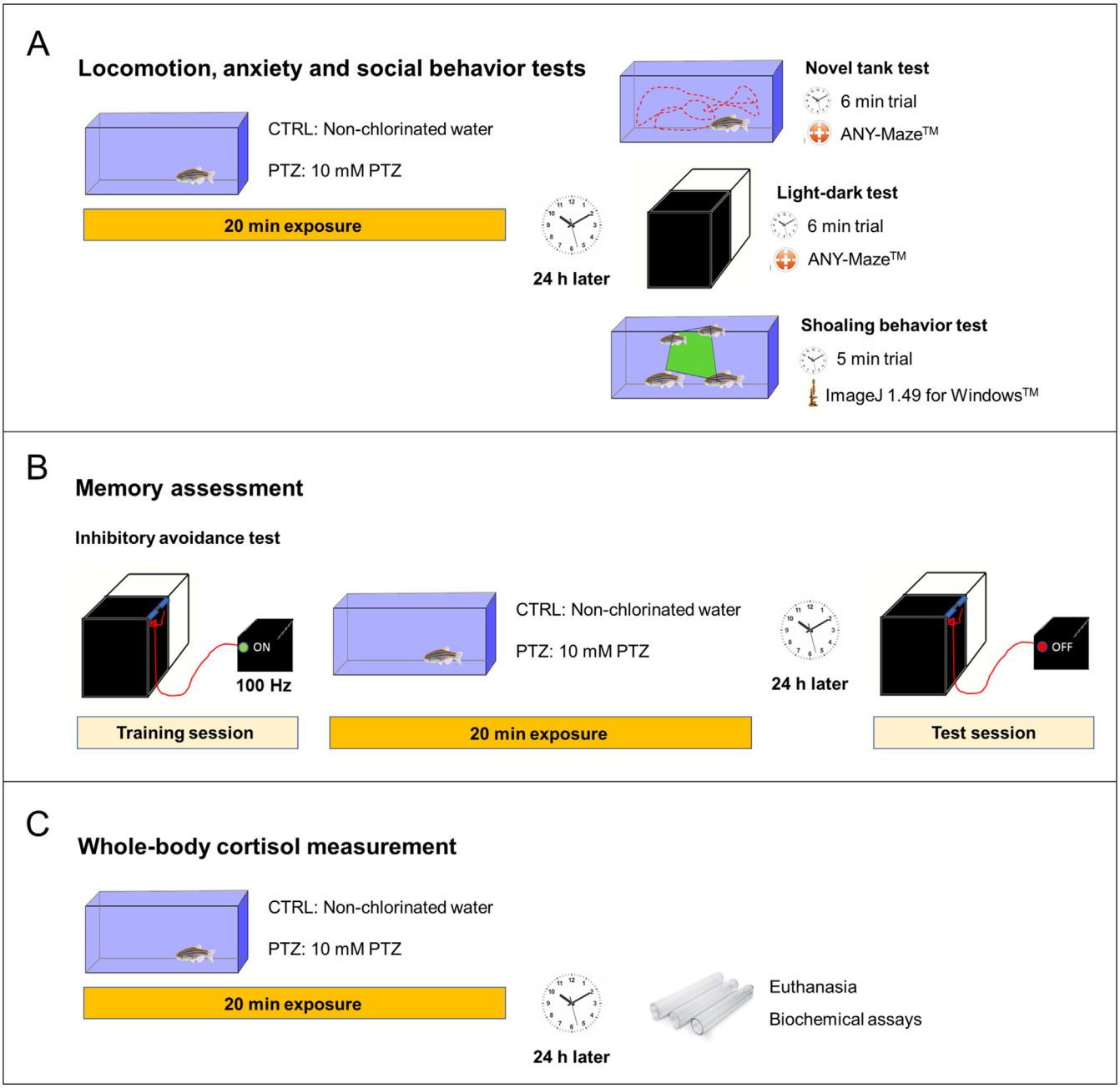

Fig. 1. Schematic representation of the experimental design and protocols used for assessing the effects of PTZ (10 mM, 20 min) on different behavioral domains of zebrafish 24 h after the exposure period. Control (CTRL) fish were kept in non-chlorinated water for the same period, in the absence of PTZ. (A) Locomotor, anxiety, and social behavior were tested using the novel tank test, light-dark test, and the shoaling test, respectively. (B) Inhibitory avoidance task, in which the effects of PTZ on consolidation memory were assessed in animals exposed immediately after the training session. (C) Whole-body cortisol measurement.

several advantageous features, such as high genetic similarities when compared to humans (Howe et al., 2013), robust pharmacological sensitivity (Goldsmith, 2004), and conserved physiological responses (Kalueff et al., 2013). Although the effects of GABAlytics PTZ and picrotoxin have been reported in zebrafish (Baraban et al., 2005; Choo et al., 2018; Wong et al., 2010b; Yang et al., 2017), the influence of epileptic seizures in different behavioral domains following recovery, in which the convulsant agent is completely out of the system, still remains poorly understood. Recent data have shown that a single PTZ-exposure increases aggression after the recovery period, suggesting the use of zebrafish models to study epilepsy-related comorbidities (Canzian et al., 2019). Moreover, PTZ-challenged zebrafish show negative impacts on spatial memory and changes in GABA and glutamate levels in the brain, supporting the evaluation of epilepsy-induced cognitive dysfunction (Choo et al., 2019; Kundap et al., 2017). Because epilepsy and its associated comorbidities are still poorly explored in this aquatic species, which represents a powerful system to assess epilepsy-related neurobehavioral phenotypes (Alfaro et al., 2011; Baraban et al., 2005; Canzian et al., 2019; Choo et al., 2018; Mussulini et al., 2013; Wong et al., 2010b), this study aimed to evaluate whether PTZ modulates distinct behavioral domains (*e.g.*, anxiety, exploratory pattern, social behavior, and memory consolidation) 24 h after a single exposure period. Moreover, since neuroendocrine responses can be associated to behavioral changes in epilepsy models (Wong et al., 2010b), the influence of whole-body cortisol levels in the behavioral responses was tested.

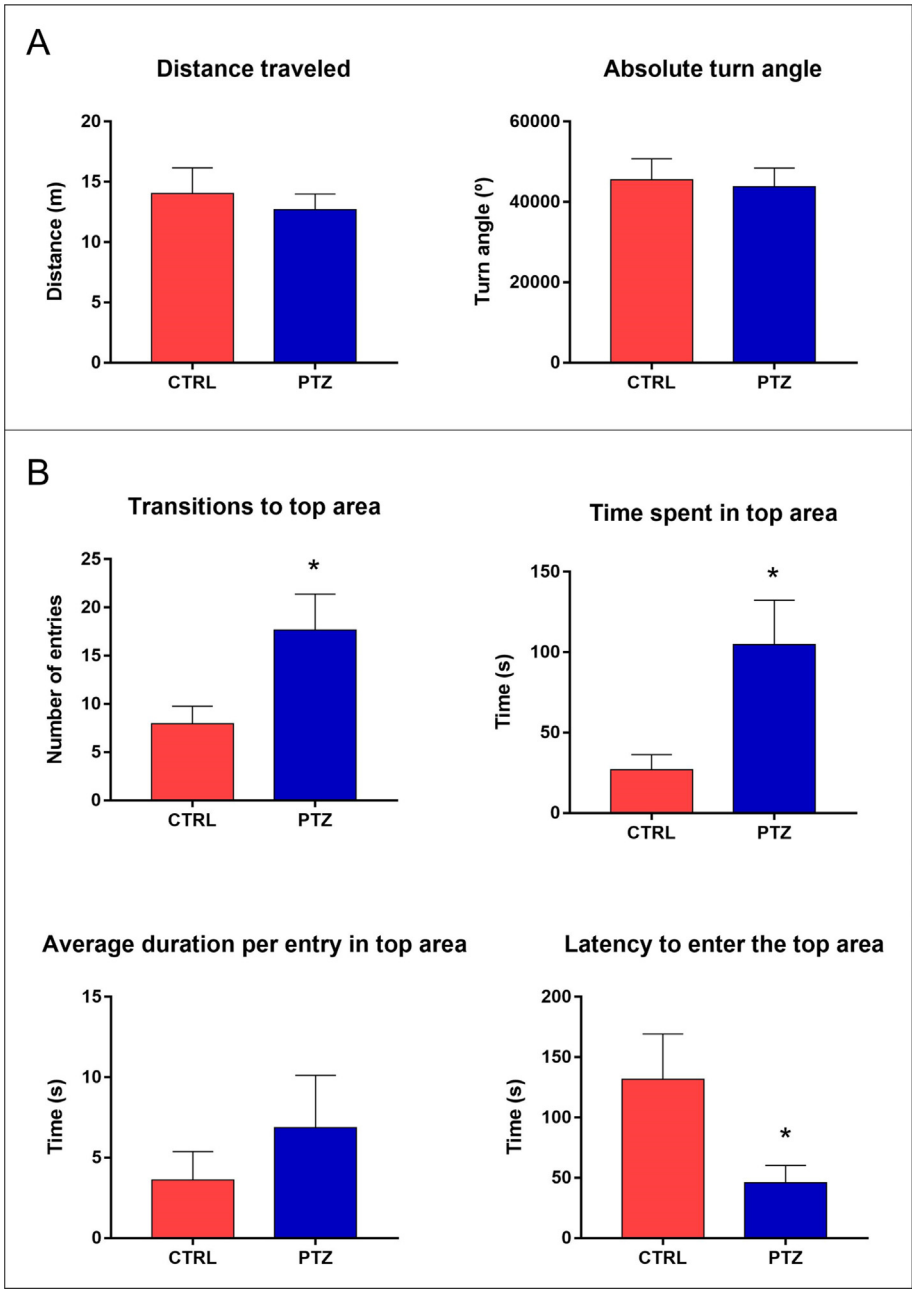

Fig. 2. Effects of PTZ on locomotion and exploratory pattern 24 h after a single exposure. **(A)** Locomotor parameters. **(B)** Vertical exploratory activity. Data were expressed as means ± S.E.M. and analyzed by unpaired Student's *t*-test. Statistical significance was set at $p \leq 0.05$ (CTRL: control; PTZ: pentylenetetrazole; $n = 12$ per group, $^{*}p < 0.05$).

2. Materials and methods

2.1. Animals

Adult zebrafish (*Danio rerio*) (4-6 months-old, ~50:50, male: female ratio, short fin phenotype) were obtained from a commercial supplier (Hobby Aquários, RS, Brazil). Subjects were kept in 40 L tanks filled with non-chlorinated water at 25 ± 2 °C and pH 7.1, conductivity at 1300–1500 μS cm^{-1}, dissolved oxygen at 6.0 ± 0.1 mg/L, total ammonia at < 0.01 mg/L, nitrate < 50 mg/L, nitrite < 0.1 mg/L, alkalinity and hardness at 75 mg/L $CaCO_3$. Importantly, we ensured that physico-chemical conditions of water were similar to those of system water throughout the experimentation to minimize stress. Illumination was provided by fluorescent lamps on a 14/10 light/dark photoperiod cycle (lights at 7:00 a.m. and off 9:00 p.m.). Fish were fed thrice daily with commercial flake fish food (alcon BASIC™, Alcon, Brazil). All experimental procedures were approved by the Ethics Commission to Animal Use of the Federal University of Santa Maria (protocol number 8707070316).

2.2. Experimental design

Animals were exposed to a single dose of PTZ (10 mM) for 20 min to induce epileptic seizures as described elsewhere (Canzian et al., 2019; Fontana et al., 2019; Mussulini et al., 2013). PTZ was added directly to the water and control fish were kept in non-chlorinated water for the same period. All animals tested in PTZ group reached scores 4 and 5, characterized by corkscrew swimming and loss of posture, respectively, described as generalized tonic-clonic seizure-like phenotypes (data not shown) (Canzian et al., 2019; Mussulini et al., 2013). After the

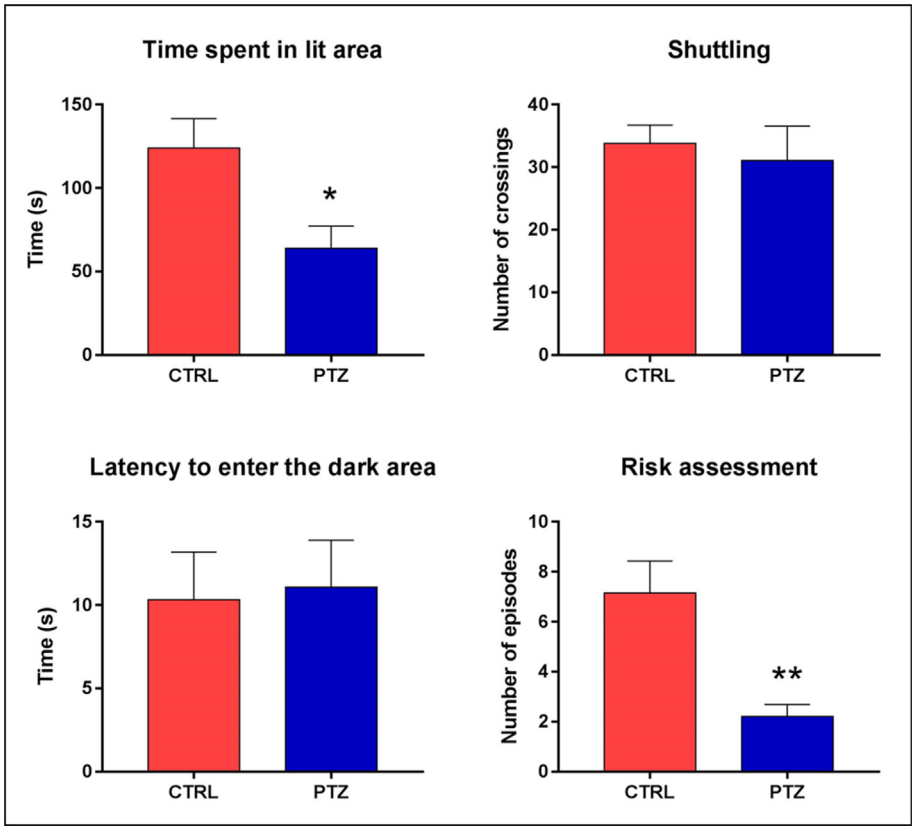

Fig. 3. Anxiety-like responses in zebrafish measured in the light-dark test 24 h after a single PTZ exposure. Data were represented as means ± S.E.M. and analyzed by unpaired Student's *t*-test. Statistical significance was set at $p \leq 0.05$ (CTRL: control; PTZ: pentylenetetrazole; $n = 12$ per group, *$p < 0.05$; **$p < 0.01$).

exposure, animals were kept in tanks filled with aerated non-chlorinated water to ensure recovery. Behaviors were measured 24 h after PTZ exposure, a period that showed prominent effects on aggression, an epilepsy-related comorbid symptom (Canzian et al., 2019). Behavioral tests were recorded between 09:00 a.m. and 4:00 p.m. and precautions to ensure representative data were taken. During the experimental procedures, fish were moved gently among the experimental apparatuses. Two batches of fish were tested for each experimental group and the tank water was replaced by non-chlorinated water across the trials. All behavioral tests were recorded in the same room, minimizing environmental influence between trials. Fig. 1 depicts a schematic representation of the experimental design.

2.3. Behavioral tests

2.3.1. Novel tank diving test

To measure locomotion and vertical activity reflecting habituation to novelty stress (Cachat et al., 2010; Rosemberg et al., 2011), fish were placed in a glass aquarium (25 cm length × 15 cm height × 25 cm width) filled with 2.0 L of non-chlorinated water, which was virtually divided into two equal horizontal areas (bottom and top) (Egan et al., 2009). Behaviors were recorded for 6 min using appropriate video-tracking software (ANY-maze™, Stoelting, CO, USA). The following endpoints were measured: distance traveled, absolute turn angle, transitions to top area, time spent in top area, average duration per entry in top area, and latency to enter the top area. Experiments were performed using $n = 12$ per group.

2.3.2. Light-dark test

To assess anxiety-like responses, the light-dark test was performed as described elsewhere (Maximino et al., 2010b; Mezzomo et al., 2016). Animals were individually placed in a rectangular glass tank (25 cm length × 15 cm height × 25 cm width) divided into two equally sized partitions with a black and white self-adhesive film externally covering the walls and floor of the tank. Behaviors were recorded for 6 min and the following endpoints were quantified: time spent in lit area, shuttling, latency to enter the dark area, and risk assessments. While the shuttling behavior expresses the transitions between compartments, which may reflect exploration in the light-dark test, risk assessments were characterized as fast (> 1 s) entries into the white section followed by re-entries intro the black section, or as partial entries in the lit area (Kalueff et al., 2013; Maximino et al., 2011). Risk assessments were measured manually by two trained observers blinded to the experimental condition (inter-rater reliability > 0.85). Experiments were performed using $n = 12$ per group.

2.3.3. Shoaling behavior test

To measure the effects of PTZ on social behavior, four fish were simultaneously placed in the test tank (25 cm length × 15 cm height × 25 cm width) for 5 min (Canzian et al., 2017). Videos were further exported to Image J 1.49 software for Windows™ and the shoaling behavior was measured using screenshots made every 15 s over the test period (total of 20 screenshots per group) (Canzian et al., 2017; Green et al., 2012). Screenshots were further calibrated to the size of the tank and each fish was marked to allow automated quantification of the proximity between the fish (inter-fish distance, nearest neighbor distance, and farthest neighbor distance), and the group dispersion (shoal area). Social interaction index was measured considering the proximity to conspecifics at a maximum distance of 3 body lengths (6 cm) (Canzian et al., 2017). Indexes vary from low cohesion ("0") to complete cohesion ("6") and the area under de curve (AUC) was used to estimate the number of social interactions. Two trained observers (inter-rater reliability > 0.85) blinded to the experimental condition analyzed the results. Experiments were performed using $n = 12$ per

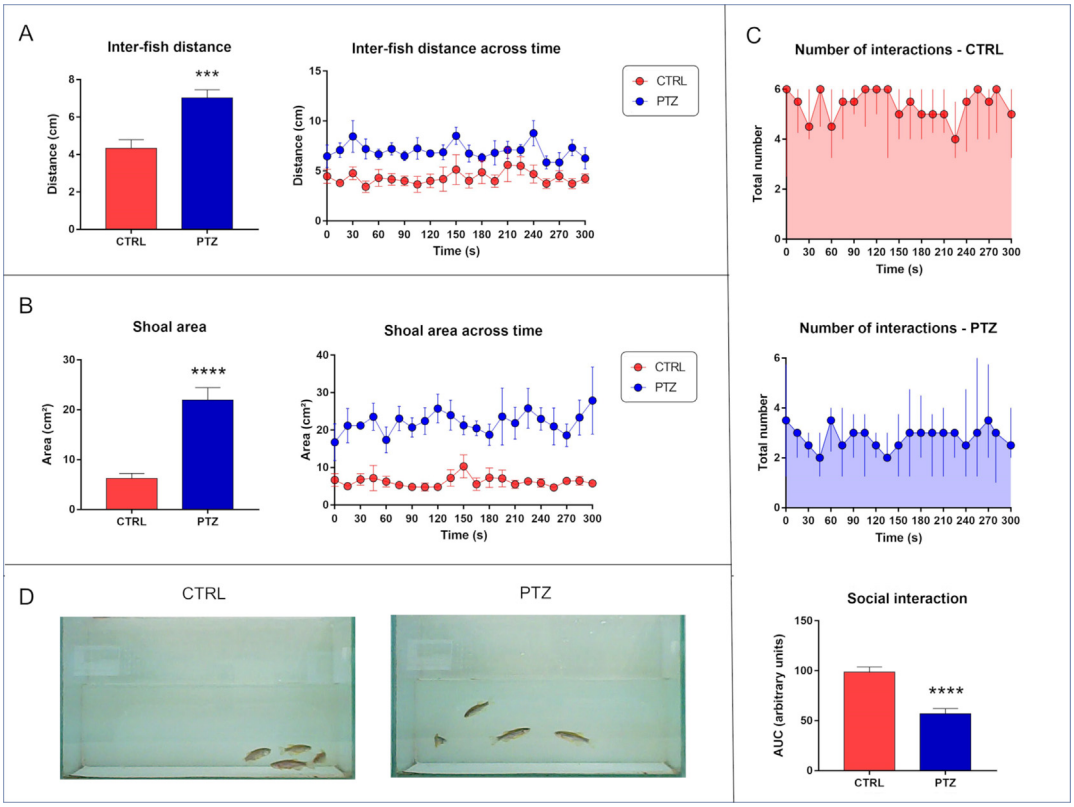

Fig. 4. PTZ disrupts shoaling behavior 24 h after a single exposure. **(A)** Temporal analysis of the inter-fish distance. **(B)** Shoal area across the 5-min trial. **(C)** Social interaction among conspecifics. **(D)** Representative images displaying the shoal cohesion of zebrafish groups. Data from inter-fish distance and shoal area were expressed as means ± S.E.M. and analyzed by repeated measures ANOVA followed by Student-Newman-Keuls multiple comparison test when necessary (temporal analyses) or by unpaired Student's *t*-test. The number of interactions across time was expressed as median ± interquartile range and the AUC was expressed as means ± S.E.M. and analyzed by unpaired Student's *t*-test. Statistical significance was set at $p \leq 0.05$ (CTRL: control; PTZ: pentylenetetrazole; $n = 8$ per group, $^{***}p < 0.005$; $^{****}p < 0.001$).

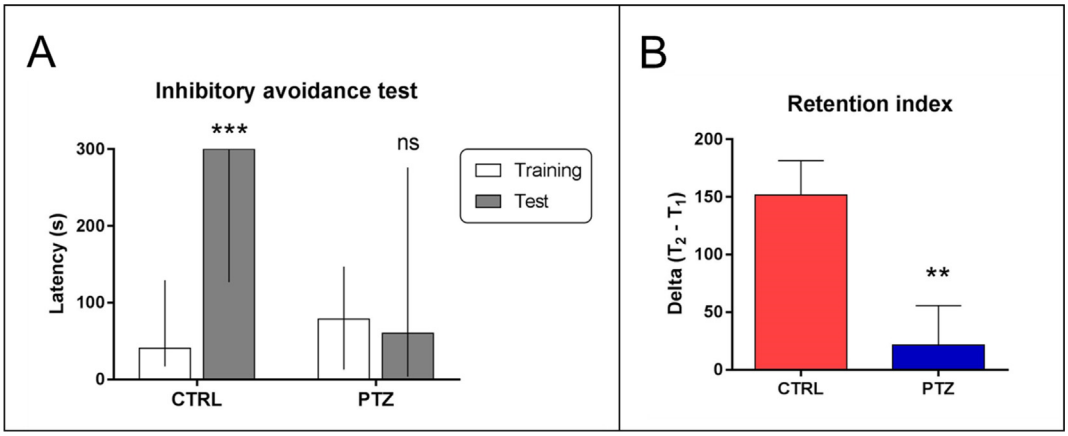

Fig. 5. Effects of PTZ on memory consolidation using the inhibitory avoidance test. **(A)** Latency to enter the dark area in training and test sessions. **(B)** Memory retention index showing negative effects of PTZ on learning performance. Data from training and test sessions were expressed as median ± interquartile range and analyzed by Wilcoxon matched-pairs signed rank test. Memory retention indexes were calculated by subtracting the latencies to enter the black side of the tank (recorded to a maximum of 300 s) in test (T_2) and training (T_1) sessions and expressed as means ± S.E.M. and analyzed by unpaired Student's *t*-test. Statistical significance was set at $p \leq 0.05$ (CTRL: control; PTZ: pentylenetetrazole; $n = 12$–14 per group, $^{**}p < 0.01$; $^{***}p < 0.005$; ns: non-significant).

group.

2.3.4. Inhibitory avoidance test

The inhibitory avoidance was performed as described elsewhere (Bertoncello et al., 2019; Blank et al., 2009; Ng et al., 2012). First, zebrafish cohorts were habituated for three days in perforated housing tanks (50 cm length × 6 cm height × 35 cm width), with similar division to each fish (6 cm × 6 cm × 6 cm), aiming to minimize the isolation stress (Maximino et al., 2018). The apparatus was a rectangular tank (30 cm length × 10 cm height × 10 cm width) filled with 1.3 L of non-chlorinated water. Tank was divided into two equally sided black and white sections separated by a manually operated opaque guillotine-type partition (10 cm × 10 cm). The black compartment contained three pairs of stain steel metal bars (1 cm diameter) spaced 5 cm within bars, connected to a 12 V stimulator. The shock frequency was set at 100 Hz (50 pulses of 5 ms every 500 ms) (Bertoncello et al.,

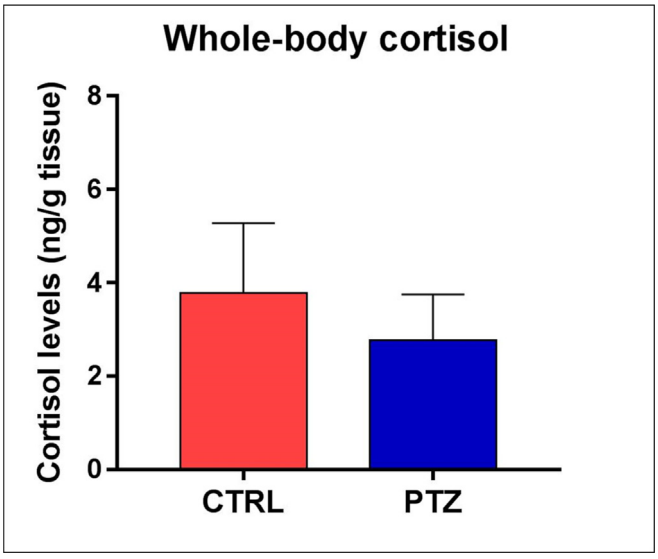

Fig. 6. PTZ does not change whole-body cortisol levels 24 h after a single exposure when compared to control (CTRL). Data were expressed as means ± S.E.M. and analyzed by unpaired Student's *t*-test. Statistical significance was set at $p \leq 0.05$ ($n = 8$ per group).

2019). At the training session, zebrafish were individually placed on the white partition of the tank. After 1 min acclimatization, the guillotine door was opened, allowing fish to enter the black side. When fish crossed to the black compartment, the sliding partition was closed and a mild electric shock (125 mA, 3 ± 0.2 V AC) was administered for 5 s. To verify whether PTZ affects memory consolidation, fish were carefully removed from the apparatus and exposed to non-chlorinated water (control) or PTZ for 20 min, immediately after the training session. Later, animals were placed in their respective perforated housing tanks. Memory retention was evaluated after 24 h similarly to the training session, except that no shock was administered. The retention index was estimated as the difference (in seconds) of latencies to enter the black side of the tank (recorded to a maximum of 300 s) in test (T_2) and training (T_1) sessions. Experiments were performed using $n = 12$–14 per group.

2.4. Cortisol extraction and analysis

Whole-body cortisol was extracted as described elsewhere (Mezzomo et al., 2019; Rosa et al., 2018). After behavioral analyses, animals ($n = 8$ per group) were weight and immediately frozen in liquid nitrogen for 10–30 s, followed by storage at −20 °C until cortisol extraction. Each fish was minced and homogenized in 1 mL phosphate buffer saline (PBS, pH 7.4). Then, samples were transferred to 2.5 mL ethyl ether, mixed for 1 min, centrifuged (800 × *g* for 10 min at 4 °C), and immediately frozen in liquid nitrogen. The portion containing ethyl ether and cortisol was transferred to another tube and completely evaporated. The cortisol fraction was resuspended in 100 µL PBS and used to measure whole-body cortisol content (EIAgen™ CORTISOL test, BioChem ImmunoSystems). Both cortisol standard curve (0.5, 2, 5, 10, 30, and 60 µg/dL) and samples were run in duplicate and data were expressed as ng cortisol/g tissue (Oliveira et al., 2014).

2.5. Statistical analysis

Normality of data and homogeneity of the variances were analyzed by Kolmogorov-Smirnov and Bartlett's tests, respectively. Data normally distributed and homoscedastic were expressed as means ± standard error of the mean (S.E.M) and further analyzed by unpaired Student's *t*-test. Nonparametric data (number of interactions and latency to enter the black compartment) were expressed median ± interquartile range. The latencies to enter the black side in training and test sessions of the inhibitory avoidance task were analyzed by Wilcoxon matched-pairs signed rank test and temporal effects on shoaling behavior were assessed using repeated measures analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparison test when necessary. Results were considered significant when $p \leq 0.05$.

3. Results

3.1. PTZ alters the exploratory pattern in the novel tank test

Fig. 2 shows the effects of PTZ on locomotion and exploratory pattern of zebrafish in the novel tank diving test 24 h after the exposure. No differences were observed in both distance traveled ($t_{(0.05;12)} = 0.5393$, $p = 0.5951$) and absolute turn angle ($t_{(0.05;12)} = 0.2414$, $p = 0.8115$) between groups (Fig. 2A). However, PTZ-exposed fish showed more transitions to the top ($t_{(0.05;12)} = 2.344$, $p = 0.0285$) and spent more time in top ($t_{(0.05;12)} = 2.666$, $p = 0.0141$) than control. Although PTZ did not change the average duration per entry in top area ($t_{(0.05;12)} = 0.8735$, $p = 0.3918$), a shorter latency to enter the top was observed ($t_{(0.05;12)} = 2.133$, $p = 0.0444$) (Fig. 2B).

3.2. PTZ evokes anxiogenic-like behavior

Fig. 3 shows the effects of PTZ on anxiety-like responses measured in the light-dark test. PTZ-exposed fish spent less time in the lit area ($t_{(0.05;12)} = 2.742$, $p = 0.0109$) and showed fewer risk assessments episodes ($t_{(0.05;12)} = 3.606$, $p = 0.0013$) than control. No differences in the latency to enter the dark area ($t_{(0.05;12)} = 0.1907$, $p = 0.8503$) and shuttling ($t_{(0.05;12)} = 0.4369$, $p = 0.665$) were verified.

3.3. PTZ disrupts shoaling behavior

Fig. 4 demonstrates the effects of PTZ on shoaling behavior. Repeated measures ANOVA yielded significant effects of treatment for both inter-fish distance ($F_{(1,14)} = 17.75$, $p = 0.0009$) and shoal area ($F_{(1,14)} = 35.14$, $p < 0.0001$). Overall, PTZ increased the inter-fish distance ($t_{(0.05;12)} = 0.4231$, $p = 0.0008$) (Fig. 4A) and the shoal area ($t_{(0.05;12)} = 5.91$, $p < 0.0001$) (Fig. 4B) compared to control. AUC analyses showed a reduced social interaction ($t_{(0.05;12)} = 5.75$, $p < 0.0001$) in PTZ-exposed fish across the 5-min trial (Fig. 4C). Fig. 4D shows representative images of control and PTZ shoals.

3.4. PTZ impairs memory consolidation

Fig. 5 shows the effects of PTZ on memory consolidation. Wilcoxon test comparison indicated that control fish showed memory retention on test session ($W = 99.00$; $p = 0.0006$), while the latencies to enter the dark compartment between training and test sessions did not differ in PTZ-exposed group ($W = 4.0$; $p = 0.9097$) (Fig. 5A). Unpaired Student's *t*-test revealed significant differences in the retention indexes, showing impaired cognition of PTZ-exposed group ($t_{(0.05,\ 12)} = 2.868$; $p = 0.0085$) in the inhibitory avoidance task (Fig. 5B).

3.5. PTZ does not alter whole-body cortisol levels

To investigate whether changes in the stress response could play a role in the behavioral responses measured, we determined whole-body cortisol levels 24 h after PTZ exposure. No significant changes were observed between control and PTZ-exposed groups ($t_{(0.05,\ 8)} = 0.5819$; $p = 0.5749$) (Fig. 6).

4. Discussion

This study characterized some behavioral phenotypes that parallel those observed in epilepsy-related comorbidities using the zebrafish as a model organism. Here, fish were challenged with PTZ at a convulsant dose (10 mM for 20 min) and different behavioral domains were tested 24 h after the exposure, a period in which PTZ is expected to get completely out of the system. For the first time, we observed that PTZ-exposed fish showed robust changes in the exploratory pattern during novelty stress, as well as anxiogenic-like responses, disrupted shoaling behavior, and impaired memory consolidation without modifications in whole-body cortisol levels 24 h after a single PTZ exposure. Because psychiatric comorbidities are predominant in epileptic patients contributing to the overall burden of disease (Di Liberto et al., 2018), these data reinforce the utility of zebrafish as a promising tool to investigate the neurobehavioral bases underlying epileptic seizures and associated comorbidities in translational neuropsychiatric research.

Mounting evidence shows that both larval and adult zebrafish display clonus-like convulsions when acutely exposed to PTZ (Baraban et al., 2005; Canzian et al., 2019; Fontana et al., 2019; Mussulini et al., 2013). PTZ antagonizes $GABA_A$ receptors, inducing ictal and interictal-like electrographic discharges, as well as increased *c-fos* expression in the CNS (Baraban et al., 2005; Pineda et al., 2011). Additionally to the oxidative stress in the brain (Fontana et al., 2019), these robust seizure-like phenotypes are accompanied by elevated whole-body cortisol levels following PTZ exposure (Wong et al., 2010b), suggesting the involvement of neuroendocrine responses and oxidant processes with epilepsy pathogenesis.

Epilepsy may trigger various comorbid conditions, including anxiety, social dysfunction, and memory deficits (Josephson and Jette, 2017; Tramoni-Negre et al., 2017). Among these comorbidities, anxiety is a psychiatric disorder that impairs millions of people worldwide (Kessler et al., 2005). Anxiety-like behaviors can be assessed in zebrafish by analyzing both habituation to novelty stress (Rosemberg et al., 2011; Wong et al., 2010a) and the preference for dark environments in detriment of bright ones (scototaxis) (Maximino et al., 2010a). Using the novel tank diving test, we verified that PTZ increased the time spent in top area and reduced the latency to enter the top 24 h after the exposure period. In the light-dark test, PTZ-exposed fish spent less time in the lit area and showed fewer risk assessment episodes. These findings suggest heightened anxiety-like behavior and a disrupted habituation to novelty stress following the recovery period. Similarly, anxiogenic substances, such as alarm pheromone, caffeine, increase scototaxis (Maximino et al., 2011; Quadros et al., 2016). Because $GABA_A$ receptors are classical pharmacological targets involved in anxiety and epilepsy (Jones-Davis and Macdonald, 2003; Lydiard, 2003), the antagonism of GABAergic receptors caused by PTZ play a role in anxiogenic-like responses (Treiman, 2001). Furthermore, genes involved in oxidant processes have been associated with anxiety-like phenotype, since the activity of antioxidant enzymes is elevated in anxious mice (Bouayed et al., 2009; Hovatta et al., 2005). These set of data corroborate previous findings, reinforcing a key role of increased anxiety levels and impaired spatial awareness in the suppression of habituation after PTZ exposure (Wong et al., 2010a).

Decreased sociability is one epileptic seizure-related comorbidity, which is a phenomenon associated to various neuropsychiatric disorders, such as autism and schizophrenia (Green, 2016; Pelphrey et al., 2004; Pinkham et al., 2008). Because the zebrafish is a social species (Oliveira, 2013), measuring the shoaling behavior is a simple protocol to evaluate the social interaction among conspecifics (Buske and Gerlai, 2011; Maaswinkel et al., 2013). Here, we observed a markedly disruption of shoaling behavior 24 h after PTZ exposure. The involvement of GABAergic system in social behavior has been postulated, since picrotoxin and PTZ administration reduces sociability of rats (File and Lister, 1984). Thus, the reduced shoaling observed herein may be occur due to antagonism of $GABA_A$ receptor, suggesting a modulatory role of epileptic seizures in zebrafish sociability.

Cognitive deficits have been recognized as another epilepsy-related comorbidity (Leeman-Markowski and Schachter, 2016). Due to the presence of a rich behavioral repertoire, which has been extensively characterized (Kalueff et al., 2013), the zebrafish is an emerging model system to study the neural bases involved in learning and memory processes (Bertoncello et al., 2019; Blank et al., 2009; Gerlai, 2016). Using the inhibitory avoidance task, we observed that PTZ impairs memory consolidation in zebrafish since exposure was performed immediately after the training session. Importantly, because distance traveled was not altered 24 h after the exposure period (as observed in the novel tank test), this response is not associated with locomotor changes, reinforcing the deleterious effects of PTZ on cognitive processes. PTZ modulates GABA and glutamate levels and induces spatial memory deficits when zebrafish are tested in a T-maze apparatus at 3 and 24 h post-challenge, supporting a modulatory role of epileptic seizures in cognition (Choo et al., 2019; Kundap et al., 2017, 2019). Because the mechanisms underlying the effects of PTZ on memory are multifactorial, changes in neurotransmitter levels and/or in the functionality of molecules involved in long-term memory processing (*e.g.*, CaMKII and cAMP) (Dong and Rosenberg, 2004; Pi et al., 2004) may play a role in the novel findings regarding the memory consolidation deficit described here.

Cortisol is the main hormone responsible for triggering stress reactions in both humans and zebrafish (Alsop and Vijayan, 2009; Baiamonte et al., 2015; Fontana et al., 2018). In zebrafish, PTZ increases whole-body cortisol levels immediately after epileptic seizures (Wong et al., 2010b). This effect on neuroendocrine stress response can be associated with the inhibitory effect on the GABAergic system, since GABA-mimetic drugs reduce the cortisol-mediated signaling (Basta-Kaim et al., 2007; Mikkelsen et al., 2008; Zhang and Liu, 2008). Based on our findings and considering the cortisol peak in zebrafish (15 min after an acute stressor) (Mezzomo et al., 2019), we hypothesize that the activation of stress response is not sustained for a 24 h period following PTZ exposure, which exclude a role of cortisol on the behavioral responses measured. Nonetheless, future studies using the recently described PTZ-induced kindling model (Kundap et al., 2019) as well a time-dependent evaluation of cortisol levels at the recovery period can be performed to explore the involvement of stress response in different behavioral domains.

5. Conclusion

In sum, our data describe multiple behavioral phenotypes that closely resemble comorbid symptoms of epilepsy in zebrafish 24 h after a single PTZ exposure. Due to the high genetic and behavioral tractability of this aquatic species (Howe et al., 2013; Kalueff et al., 2013), our data foster markedly the utility of zebrafish models for studying the neurobiological bases of epilepsy-related comorbidities in a translational perspective. Finally, combining the high-throughput potential of zebrafish (Choo et al., 2019; Gerlai, 2010) and the availability of automated video-tracking systems for behavioral neurophenotyping (Cachat et al., 2011), our data help validate the use of alternative fish species to investigate the fundamental neural mechanisms involved in epilepsy-related comorbidities.

Conflicts of interest

The authors declare that no competing interests exist.

Acknowledgments

We recognize the financial support and fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). J.C. was recipient of fellowship from CNPq. T.E.M., F.F., P.M.,

B.D.F., and F.V.C. were recipients of fellowship from CAPES. D.B.R. was recipient of CNPq research productivity grant (305051/2018-0) and his work is also supported by the PROEX/CAPES (process number 23038.004173/2019-93) and PRONEM/FAPERGS (process number 16/2551-0000248-7) fellowship grants. All authors contributed to the preparation of the manuscript and approved its final version. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Alfaro, J.M., Ripoll-Gomez, J., Burgos, J.S., 2011. Kainate administered to adult zebrafish causes seizures similar to those in rodent models. Eur. J. Neurosci. 33 (7), 1252–1255.

Alsop, D., Vijayan, M., 2009. The zebrafish stress axis: molecular fallout from the teleost-specific genome duplication event. Gen. Comp. Endocrinol. 161 (1), 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 (4), e0124488.

Baraban, S.C., Taylor, M.R., Castro, P.A., Baier, H., 2005. Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. Neuroscience 131 (3), 759–768.

Basta-Kaim, A., Budziszewska, B., Leskiewicz, M., Regulska, M., Otczyk, M., Kubera, M., Jagla, G., Nowak, W., Luszczki, J.J., Czuczwar, S.J., Lason, W., 2007. Effects of some new antiepileptic drugs and progabide on glucocorticoid receptor-mediated gene transcription in LMCAT cells. Pharmacol. Rep. 59 (5), 531–537.

Bertoncello, K.T., Muller, T.E., Fontana, B.D., Franscescon, F., Filho, G.L.B., Rosemberg, D.B., 2019. Taurine prevents memory consolidation deficits in a novel alcohol-induced blackout model in zebrafish. Prog Neuropsychopharmacol Biol Psychiatry 93, 39–45.

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 (4), 529–534.

Bouayed, J., Rammal, H., Soulimani, R., 2009. Oxidative stress and anxiety: relationship and cellular pathways. Oxid Med Cell Longev 2 (2), 63–67.

Buske, C., Gerlai, R., 2011. Shoaling develops with age in Zebrafish (*Danio rerio*). Prog Neuropsychopharmacol Biol Psychiatry 35 (6), 1409–1415.

Cachat, J., Stewart, A., Grossman, L., Gaikwad, S., Kadri, F., Chung, K.M., 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.V., 2010. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat. Protoc. 5 (11), 1786–1799.

Cachat, J., Stewart, A., Utterback, E., Hart, P., Gaikwad, S., Wong, K., Kyzar, E., Wu, N., Kalueff, A.V., 2011. Three-dimensional neurophenotyping of adult zebrafish behavior. PLoS One 6 (3), e17597.

Canzian, J., Fontana, B.D., Quadros, V.A., Muller, T.E., Duarte, T., Rosemberg, D.B., 2019. Single pentylenetetrazole exposure increases aggression in adult zebrafish at different time intervals. Neurosci. Lett. 692, 27–32.

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.

Choo, B.K.M., Kundap, U.P., Johan Arief, M.F.B., Kumari, Y., Yap, J.L., Wong, C.P., Othman, I., Shaikh, M.F., 2019. Effect of newer anti-epileptic drugs (AEDs) on the cognitive status in pentylenetetrazol induced seizures in a zebrafish model. Prog Neuropsychopharmacol Biol Psychiatry 92, 483–493.

Choo, B.K.M., Kundap, U.P., Kumari, Y., Hue, S.M., Othman, I., Shaikh, M.F., 2018. *Orthosiphon stamineus* leaf extract affects TNF-alpha and seizures in a zebrafish model. Front. Pharmacol. 9, 139.

Dhir, A., 2012. Pentylenetetrazol (PTZ) kindling model of epilepsy. Curr Protoc Neurosci Chapter 9 Unit9 37.

Di Liberto, V., van Dijk, R.M., Brendel, M., Waldron, A.M., Moller, C., Koska, I., Seiffert, I., Gualtieri, F., Gildehaus, F.J., von Ungern-Sternberg, B., Lindner, M., Ziegler, S., Palme, R., Hellweg, R., Gass, P., Bartenstein, P., Potschka, H., 2018. Imaging correlates of behavioral impairments: an experimental PET study in the rat pilocarpine epilepsy model. Neurobiol. Dis. 118, 9–21.

Dong, Y., Rosenberg, H.C., 2004. Brief seizure activity alters Ca2+/calmodulin dependent protein kinase II dephosphorylation and subcellular distribution in rat brain for several hours. Neurosci. Lett. 357 (2), 95–98.

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 (1), 38–44.

Engel Jr., J., Pitkanen, A., Loeb, J.A., Dudek, F.E., Bertram 3rd, E.H., Cole, A.J., Moshe, S.L., Wiebe, S., Jensen, F.E., Mody, I., Nehlig, A., Vezzani, A., 2013. Epilepsy biomarkers. Epilepsia 54 (Suppl. 4), 61–69.

File, S.E., Lister, R.G., 1984. Do the reductions in social interaction produced by picrotoxin and pentylenetetrazole indicate anxiogenic actions? Neuropharmacology 23 (7A), 793–796.

Fontana, B.D., Mezzomo, N.J., Kalueff, A.V., Rosemberg, D.B., 2018. The developing utility of zebrafish models of neurological and neuropsychiatric disorders: a critical review. Exp. Neurol. 299 (Pt A), 157–171.

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 pentylenetetrazole-induced behavioral and neurochemical changes in zebrafish. Mol. Neurobiol. 56 (1), 583–594.

Gerlai, R., 2010. High-throughput behavioral screens: the first step towards finding genes involved in vertebrate brain function using zebrafish. Molecules 15 (4), 2609–2622.

Gerlai, R., 2016. Learning and memory in zebrafish (*Danio rerio*). Methods Cell Biol. 134, 551–586.

Goldsmith, P., 2004. Zebrafish as a pharmacological tool: the how, why and when. Curr. Opin. Pharmacol. 4 (5), 504–512.

Green, J., Collins, C., Kyzar, E.J., Pham, M., Roth, A., Gaikwad, S., Cachat, J., Stewart, A.M., Landsman, S., Grieco, F., Tegelenbosch, R., Noldus, L.P., Kalueff, A.V., 2012. Automated high-throughput neurophenotyping of zebrafish social behavior. J. Neurosci. Methods 210 (2), 266–271.

Green, M.F., 2016. Impact of cognitive and social cognitive impairment on functional outcomes in patients with schizophrenia. J. Clin. Psychiatry 77 (Suppl. 2), 8–11.

Henbid, M.T., Marks, W.N., Collins, M.J., Cain, S.M., Snutch, T.P., Howland, J.G., 2017. Sociability impairments in genetic absence epilepsy rats from strasbourg: reversal by the T-type calcium channel antagonist Z944. Exp. Neurol. 296, 16–22.

Hovatta, I., Tennant, R.S., Helton, R., Marr, R.A., Singer, O., Redwine, J.M., Ellison, J.A., Schadt, E.E., Verma, I.M., Lockhart, D.J., Barlow, C., 2005. Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. Nature 438 (7068), 662–666.

Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J.C., Koch, R., Rauch, G.J., White, S., Chow, W., Kilian, B., Quintais, L.T., Guerra-Assuncao, J.A., Zhou, Y., Gu, Y., Yen, J., Vogel, J.H., Eyre, T., Redmond, S., Banerjee, R., Chi, J., Fu, B., Langley, E., Maguire, S.F., Laird, G.K., Lloyd, D., Kenyon, E., Donaldson, S., Sehra, H., Almeida-King, J., Loveland, J., Trevanion, S., Jones, M., Quail, M., Willey, D., Hunt, A., Burton, J., Sims, S., McLay, K., Plumb, B., Davis, J., Clee, C., Oliver, K., Clark, R., Riddle, C., Elliot, D., Threadgold, G., Harden, G., Ware, D., Begum, S., Mortimore, B., Kerry, G., Heath, P., Phillimore, B., Tracey, A., Corby, N., Dunn, M., Johnson, C., Wood, J., Clark, S., Pelan, S., Griffiths, G., Smith, M., Glithero, R., Howden, P., Barker, N., Lloyd, C., Stevens, C., Harley, J., Holt, K., Panagiotidis, G., Lovell, J., Beasley, H., Henderson, C., Gordon, D., Auger, K., Wright, D., Collins, J., Raisen, C., Dyer, L., Leung, K., Robertson, L., Ambridge, K., Leongamornlert, D., McGuire, S., Gilderthorp, R., Griffiths, C., Manthravadi, D., Nichol, S., Barker, G., Whitehead, S., Kay, M., Brown, J., Murnane, C., Gray, E., Humphries, M., Sycamore, N., Barker, D., Saunders, D., Wallis, J., Babbage, A., Hammond, S., Mashreghi-Mohammadi, M., Barr, L., Martin, S., Wray, P., Ellington, A., Matthews, N., Ellwood, M., Woodmansey, R., Clark, G., Cooper, J., Tromans, A., Grafham, D., Skuce, C., Pandian, R., Andrews, R., Harrison, E., Kimberley, A., Garnett, J., Fosker, N., Hall, R., Garner, P., Kelly, D., Bird, C., Palmer, S., Gehring, I., Berger, A., Dooley, C.M., Ersan-Urun, Z., Eser, C., Geiger, H., Geisler, M., Karotki, L., Kirn, A., Konantz, J., Konantz, M., Oberlander, M., Rudolph-Geiger, S., Teucke, M., Lanz, C., Raddatz, G., Osoegawa, K., Zhu, B., Rapp, A., Widaa, S., Langford, C., Yang, F., Schuster, S.C., Carter, N.P., Harrow, J., Ning, Z., Herrero, J., Searle, S.M., Enright, A., Geisler, R., Plasterk, R.H., Lee, C., Westerfield, M., de Jong, P.J., Zon, L.I., Postlethwait, J.H., Nusslein-Volhard, C., Hubbard, T.J., Roest Crollius, H., Rogers, J., Stemple, D.L., 2013. The zebrafish reference genome sequence and its relationship to the human genome. Nature 496 (7446), 498–503.

Huang, R.Q., Bell-Horner, C.L., Dibas, M.I., Covey, D.F., Drewe, J.A., Dillon, G.H., 2001. Pentylenetetrazole-induced inhibition of recombinant gamma-aminobutyric acid type A (GABA(A)) receptors: mechanism and site of action. J. Pharmacol. Exp. Ther. 298 (3), 986–995.

Hunter, M.B., Yoong, M., Sumpter, R.E., Verity, K., Shetty, J., McLellan, A., Jones, J., Quigley, A., Tallur, K.K., Chin, R.F.M., 2019. Neurobehavioral problems in children with early-onset epilepsy: a population-based study. Epilepsy Behav. 93, 87–93.

Jones-Davis, D.M., Macdonald, R.L., 2003. GABA(A) receptor function and pharmacology in epilepsy and status epilepticus. Curr. Opin. Pharmacol. 3 (1), 12–18.

Josephson, C.B., Jette, N., 2017. Psychiatric comorbidities in epilepsy. Int. Rev. Psychiatry 29 (5), 409–424.

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., Neuhauss, 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 (1), 70–86.

Kessler, R.C., Chiu, W.T., Demler, O., Merikangas, K.R., Walters, E.E., 2005. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the national comorbidity survey replication. Arch. Gen. Psychiatr. 62 (6), 617–627.

Kundap, U.P., Kumari, Y., Othman, I., Shaikh, M.F., 2017. Zebrafish as a model for epilepsy-induced cognitive dysfunction: a pharmacological, biochemical and behavioral approach. Front. Pharmacol. 8, 515.

Kundap, U.P., Paudel, Y.N., Kumari, Y., Othman, I., Shaikh, M.F., 2019. Embelin prevents seizure and associated cognitive impairments in a pentylenetetrazole-induced kindling zebrafish model. Front. Pharmacol. 10, 315.

Leeman-Markowski, B.A., Schachter, S.C., 2016. Treatment of cognitive deficits in epilepsy. Neurol. Clin. 34 (1), 183–204.

Lydiard, R.B., 2003. The role of GABA in anxiety disorders. J. Clin. Psychiatry 64 (Suppl. 3), 21–27.

Maaswinkel, H., Zhu, L., Weng, W., 2013. Assessing social engagement in heterogeneous groups of zebrafish: a new paradigm for autism-like behavioral responses. PLoS One 8 (10), e75955.

Maximino, C., da Silva, A.W., Gouveia Jr., A., Herculano, A.M., 2011. Pharmacological

analysis of zebrafish (*Danio rerio*) scototaxis. Prog Neuropsychopharmacol Biol Psychiatry 35 (2), 624–631.
Maximino, C., Marques de Brito, T., Dias, C.A., Gouveia Jr., A., Morato, S., 2010a. Scototaxis as anxiety-like behavior in fish. Nat. Protoc. 5 (2), 209–216.
Maximino, C., Marques de Brito, T., Dias, C.A., Gouveia, A.J., Morato, S., 2010b. Scototaxis as anxiety-like behavior in fish. Nat. Protoc. 5 (2), 209–216.
Maximino, C., Meinerz, D.L., Fontana, B.D., Mezzomo, N.J., Stefanello, F.V., de, S.P.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. Process. 149, 35–42.
Mazarati, A.a.S., R., 2016. Common mechanisms UnderlyingEpileptogenesis and the co-morbiditiesof epilepsy. Cold Spring Harb Perspect Med 6 (7), a022798.
Mezzomo, N.J., Fontana, B.D., Muller, T.E., Duarte, T., Quadros, V.A., Canzian, J., Pompermaier, A., Soares, S.M., Koakoski, G., Loro, V.L., Rosemberg, D.B., Barcellos, L.J.G., 2019. Taurine modulates the stress response in zebrafish. Horm. Behav. 109, 44–52.
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.
Mikkelsen, J.D., Bundzikova, J., Larsen, M.H., Hansen, H.H., Kiss, A., 2008. GABA regulates the rat hypothalamic-pituitary-adrenocortical axis via different GABA-A receptor alpha-subtypes. Ann. N. Y. Acad. Sci. 1148, 384–392.
Murrough, J.W., Yaqubi, S., Sayed, S., Charney, D.S., 2015. Emerging drugs for the treatment of anxiety. Expert Opin. Emerg. Drugs 20 (3), 393–406.
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 (1), e54515.
Ng, M.C., Hsu, C.P., Wu, Y.J., Wu, S.Y., Yang, Y.L., Lu, K.T., 2012. Effect of MK-801-induced impairment of inhibitory avoidance learning in zebrafish via inactivation of extracellular signal-regulated kinase (ERK) in telencephalon. Fish Physiol. Biochem. 38 (4), 1099–1106.
Oliveira, R.F., 2013. Mind the fish: zebrafish as a model in cognitive social neuroscience. Front. Neural Circuits 7, 131.
Oliveira, T.A., Koakoski, G., da Motta, A.C., Piato, A.L., Barreto, R.E., Volpato, G.L., Barcellos, L.J., 2014. Death-associated odors induce stress in zebrafish. Horm. Behav. 65 (4), 340–344.
Pelphrey, K., Adolphs, R., Morris, J.P., 2004. Neuroanatomical substrates of social cognition dysfunction in autism. Ment. Retard. Dev. Disabil. Res. Rev. 10 (4), 259–271.
Pi, X., Lee, J., Li, F., Rosenberg, H.C., 2004. Decreased expression of brain cAMP response element-binding protein gene following pentylenetetrazol seizure. Brain Res Mol Brain Res 127 (1–2), 60–67.
Pineda, R., Beattie, C.E., Hall, C.W., 2011. Recording the adult zebrafish cerebral field potential during pentylenetetrazole seizures. J. Neurosci. Methods 200 (1), 20–28.
Pinkham, A.E., Hopfinger, J.B., Pelphrey, K.A., Piven, J., Penn, D.L., 2008. Neural bases for impaired social cognition in schizophrenia and autism spectrum disorders. Schizophr. Res. 99 (1–3), 164–175.
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.
Rosa, L.V., Ardais, A.P., Costa, F.V., Fontana, B.D., Quadros, V.A., Porciuncula, L.O., Rosemberg, D.B., 2018. Different effects of caffeine on behavioral neurophenotypes of two zebrafish populations. Pharmacol. Biochem. Behav. 165, 1–8.
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 (5), e19397.
Sierra-Paredes, G., Sierra-Marcuno, G., 2007. Extrasynaptic GABA and glutamate receptors in epilepsy. CNS Neurol. Disord. - Drug Targets 6 (4), 288–300.
Singh, A., Trevick, S., 2016. The epidemiology of global epilepsy. Neurol. Clin. 34 (4), 837–847.
Tellez-Zenteno, J.F., Patten, S.B., Jette, N., Williams, J., Wiebe, S., 2007. Psychiatric comorbidity in epilepsy: a population-based analysis. Epilepsia 48 (12), 2336–2344.
Tramoni-Negre, E., Lambert, I., Bartolomei, F., Felician, O., 2017. Long-term memory deficits in temporal lobe epilepsy. Rev. Neurol. (Paris) 173 (7–8), 490–497.
Treiman, D.M., 2001. GABAergic mechanisms in epilepsy. Epilepsia 42 (Suppl. 3), 8–12.
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., 2010a. Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). Behav. Brain Res. 208 (2), 450–457.
Wong, K., Stewart, A., Gilder, T., Wu, N., Frank, K., Gaikwad, S., Suciu, C., Dileo, J., Utterback, E., Chang, K., Grossman, L., Cachat, J., Kalueff, A.V., 2010b. Modeling seizure-related behavioral and endocrine phenotypes in adult zebrafish. Brain Res. 1348, 209–215.
Yang, X., Lin, J., Peng, X., Zhang, Q., Zhang, Y., Guo, N., Zhou, S., Li, Q., 2017. Effects of picrotoxin on zebrafish larvae behaviors: a comparison study with PTZ. Epilepsy Behav. 70 (Pt A), 224–231.
Zhang, S.W., Liu, Y.X., 2008. Changes of serum adrenocorticotropic hormone and cortisol levels during sleep seizures. Neurosci Bull 24 (2), 84–88.
Zhu, K., Yuan, B., Hu, M., Li, C.J., Xu, J.H., Feng, G.F., Liu, Y., Liu, J.X., 2018. Ablation of aberrant neurogenesis fails to attenuate cognitive deficit of chronically epileptic mice. Epilepsy Res. 142, 1–8.