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Anxiety-related behavioral responses of pentylenetetrazole-treated zebrafish larvae to light-dark transitions☆

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ABSTRACT

Pentylenetetrazole (PTZ), γ-aminobutyrate (GABA) antagonist, is a convulsant drug, known to induce anxiety and seizures in zebrafish. Changes in the mobility of zebrafish under light-dark transitions reflect anxiety level, serving as a useful behavioral readout. The effects of PTZ treatment have yet to be assayed in this manner. Zebrafish larvae (AB strain) at both 5 dpf (days post-fertilization) and 7 dpf were treated with different concentrations of PTZ. General locomotor activity and thigmotaxis were analyzed under continuous illumination (normal conditions) or alternating light-dark cycles (stressful conditions). Zebrafish larvae of 5 dpf and 7 dpf exhibited different sensitivities to PTZ. Anxiety level, measured in terms of response to illumination transitions under the influence of PTZ, demonstrated contrasting tendencies. Dark-light transitions dramatically increased the locomotor activity of zebrafish larvae receiving 8 mM PTZ which was indicative of anxiety. This study suggests that PTZ increases the susceptibility by activating the neuron, which perhaps makes light change easier to influence the anxiety level of larvae. We provide useful evidence for putative anti-anxiety drug screening.

1. Introduction

Zebrafish offer a unique combination of genetic tractability, close homology with higher vertebrates and accessible experimental methodologies, such as optical imaging, behavioral analysis, and electrophysiological recording. It has become a useful model system of human disease with which to study abnormal neurological and behavioral activity (Blaser and Vira, 2014).

At present, the zebrafish is emerging as a promising model organism for experimental studies of stress and anxiety (Egan et al., 2009). One of the most popular tests of anxiety in the rat is an open field exploration task, which is readily adaptable to zebrafish and has been used widely (Blaser and Gerlai, 2006). Locomotor activity and thigmotaxis are two important parameters indicative of anxiety in zebrafish. In normal zebrafish, locomotion and thigmotaxis increased in the context of elevated anxiety level. A light-dark emergence task is also used to measure anxiety in the rat, in which the rat chooses between a dark or bright chamber, the former suggesting heightened anxiety level (Arrant et al., 2013). The 'light-dark emergence' test means the animal can choose between a light and a dark chamber when the emergence appears. This task would appear readily adaptable to zebrafish (Belzung and Griebel, 2001, Ramcharitar and Ibrahim, 2013). The Light-dark forced transitions means that the light or dark status is not selective, here means light transiting to dark or dark transiting to light. Light-dark transitions increase locomotor activity in zebrafish, indicative of increased anxiety. Locomotion and thigmotaxis have been used to characterize anxiety under different light conditions (Blaser et al., 2010; Maximino et al., 2010). Pentylenetetrazole (PTZ), γ-aminobutyrate (GABA) antagonist, is a chemical convulsant that can generate clonic or tonic–clonic seizures in humans and other animals (Baraban et al., 2005; Mussulini et al., 2013). PTZ has varied, dosage-dependent effects on behavior and cerebral metabolism (Baraban et al., 2005, Hewapathirane et al., 2008; Wong et al., 2010). Recent work has demonstrated the use of zebrafish larvae as a model of bipolar disorder by treating with sub-convulsive concentrations of PTZ (Ellis and Soanes, 2012, Stewart et al., 2011). Another study reported hyperactivity induced by PTZ treatment. Locomotor activity and thigmotaxis have been shown to increase as a result of PTZ treatment (Ellis et al., 2012). In the context of a light-dark transition, PTZ-treated zebrafish larvae exhibited a reversed pattern of activity compared with controls (Ellis et al., 2012). Whereas, changes in anxiety level after PTZ treatment under

Abbreviations: GABA, γ-aminobutyrate; PTZ, pentylenetetrazole; dpf, day post-fertilization.

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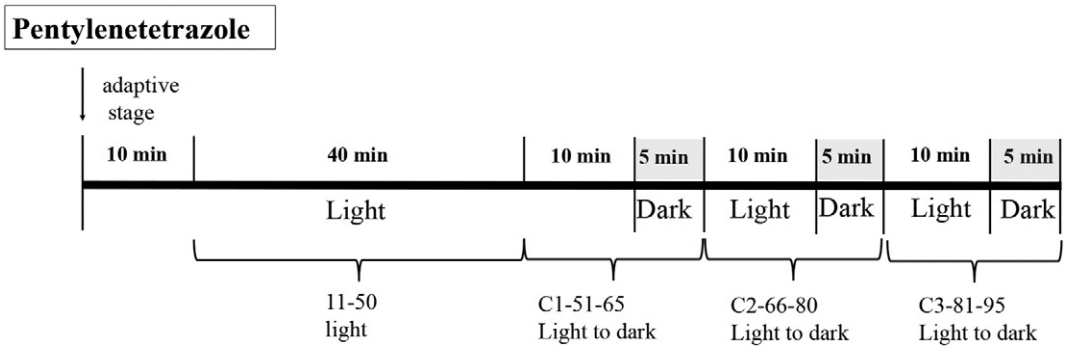

Fig. 1. Experimental format. Each experiment lasts 95 min: 1 h of continuous light and 35 min of light-dark transitions, alternating between 5 min of dark and 10 min of light. Quantification of zebrafish larvae locomotion was performed using the tracking mode of Zebralab software with recorded videos.

different illuminations have not yet been clearly demonstrated and it is still unclear how the behavior appears. Here, we utilize a dark-light transition (Emran et al., 2008; Enkel et al., 2013) to explore anxiety-related behavioral changes in zebrafish in the context of varied PTZ treatment concentrations. Locomotor activity (Irons et al., 2010; MacPhail et al., 2009) and thigmotaxis (Schnorr et al., 2012) varied depending on treatment dose, suggesting PTZ-dependent fluctuation of anxiety level.

2. Materials and methods

2.1. Zebrafish breeding

Zebrafish (AB strain) were maintained at 28.5 °C according to the Zebrafish Book. (http://zfin.org/zf_info/zfbook/zfbk.html) Fish were kept on a 14-hour light and 10-hour dark cycle (lights on at 08:00, lights off at 22:00). Eggs were obtained by natural spawning and were raised in an incubator at 28.5 °C from birth to 5 dpf or 7 dpf, as per previously published methods (Kimmel et al., 1995). Eggs and larvae were kept under the same lighting schedule as adult zebrafish.

2.2. Drugs

PTZ (Sigma-Aldrich; P6500, St. Louis, MO) was reconstituted at 32 mM in stock solution with sterilized water and frozen at −80 °C. PTZ working solution was freshly diluted from stock to appropriate concentrations with zebrafish system water prior to experiments.

2.3. Behavioral tests

All behavioral tests were performed in a room at ambient temperature (28.5 °C). The room was humidified to minimize the evaporation of water in the testing wells. Behavioral tests were carried out in 24-well plates with zebrafish larvae (5 dpf and 7 dpf). We selected the 5 and 7 dpf age for experiments according to the development characteristics of the zebrafish larvae. At 5 dpf, the larvae period, the early larvae gradually begins to swim about actively, and moves its jaws, opercular flaps, pectoral fins, and eyes. These developments produce swift escape responses and herald respiration, the seeking of prey, and feeding, whereas before this stage, during the hatching period the embryo is usually at rest. As early as 7 dpf, a stage late in larval development, most morphogenesis and primary neurogenesis is complete. The larvae were obtained from group mating and were randomly assigned either to the 5 dpf or the 7 dpf group. Experiments were arranged such that all groups were equally present in the 24-well plates in order to avoid any inter-treatment variations due to differences in experimental timing (Li et al., 2014).

The zebrafish larvae were carefully transferred to a 24-well plate with a single larva in each well; the inner diameter of the well is 18 mm. Excessive fluid was removed and 500 μL of fresh system water was immediately loaded into each well. Subsequently, 500 μL of PTZ working solution was quickly added into the wells such that each well contained 1 mL liquid. The substantial volume of the PTZ working solution ensured a good mixture of liquid in the wells. The final PTZ concentrations tested were 0 mM (control), 1 mM, 2 mM, 4 mM, 8 mM and 16 mM. The plate was then placed into a Zebrabox (ViewPoint Life Sciences), equipped to record video of zebrafish larvae activity. An infrared camera/light was used for recording automatically. We had detected the actual light level using illuminometer. The full light strength is 100 lx. The larvae were given a 10 min acclimation period with illumination, followed by a 40 min continuous illumination period to study spontaneous activity. Three 15-minute cycles followed (10 minute illumination followed by 5 minute dark, i.e., illumination off), in order to examine responses to changes in lighting conditions under the influence of PTZ. Each experimental session lasted for 95 min, including the acclimation period (Fig. 1).

Locomotor activity was quantified with Zebralab software. Videos were recorded at 25 fps and pooled into 1-minute time bin. The detection threshold was set at 25, an arbitrary level that allowed the software to accurately detect larval movement. Because the total distance traveled was the metric used for analysis, the thresholds for both inactive (no locomotion activity) and high (high locomotion activity) were set to 0 cm/s. A round-shaped center arena that occupied half of the area of a single well was defined in each well. The distance moved by the larvae in the whole well was acquired for the analysis of general locomotion activities. Measurements of the distance moved and the time spent in the center arena were acquired for the analysis of thigmotaxis behavior.

Fig. 2. Distance traveled by zebrafish larvae in the whole well under continuous illumination followed by alternating light-dark cycles. The distance traveled by zebrafish larvae in each 1-minute time bin was plotted for the course of the experiment. Experiments were performed at 5 dpf (A, B, C, D, E) and 7 dpf (F, G, H, I, J). The horizontal axis denotes the progression of the experiment. The vertical axis denotes the distance (millimeters) traveled by larvae in each 1-minute time bin. Data are presented as the mean ± SEM, n = 32 animals per group. To better visualize the data, the control group was plotted in each panel with a single PTZ concentration group. Therefore, the controls are the same across all panels with the zebrafish larvae at the same developmental stage. PTZ concentration information is marked on the top right corner of each panel.