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Effect of acute ethanol administration on zebrafish tail-beat motion

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ABSTRACT

Zebrafish is becoming a species of choice in neurobiological and behavioral studies of alcohol-related disorders. In these efforts, the activity of adult zebrafish is typically quantified using indirect activity measures that are either scored manually or identified automatically from the fish trajectory. The analysis of such activity measures has produced important insight into the effect of acute ethanol exposure on individual and social behavior of this vertebrate species. Here, we leverage a recently developed tracking algorithm that reconstructs fish body shape to investigate the effect of acute ethanol administration on zebrafish tail-beat motion in terms of amplitude and frequency. Our results demonstrate a significant effect of ethanol on the tail-beat amplitude as well as the tail-beat frequency, both of which were found to robustly decrease for high ethanol concentrations. Such a direct measurement of zebrafish motor functions is in agreement with evidence based on indirect activity measures, offering a complementary perspective in behavioral screening.

Introduction

Zebrafish is increasingly used as an animal model to study the effect of ethanol administration on individual and social behavior (Echevarria, Toms, & Jouandot, 2011). In these experiments, zebrafish behavior is typically studied using measures of activity that are scored manually or computed automatically from the trajectory of the fish. While previous experiments on acute ethanol treatment have highlighted significant effects on adult zebrafish locomotion (Blazina, Vianna, & Lara, 2013; Cianca, Bartolini, Porfiri, & Macrì, 2013; Gerlai, Lahav, Guo, & Rosenthal, 2000; Ladu, Butail, Macrì, & Porfiri, 2014; Mathur & Guo, 2011; Spinello, Macrì, & Porfiri, 2013), a lack of high-throughput methods to measure zebrafish tail-beat motion has hindered a thorough understanding of the modalities in which ethanol impinges on motor functions.

Motor functions have been extensively investigated in larval zebrafish in terms of tail-beat motion during escape and predatory avoidance, prey capture, and swimming (Ikeda et al., 2013; McClenahan, Troup, & Scott, 2012; Pham et al., 2012; Thorsen, Cassidy, & Hale, 2004; Trivedi & Bollmann, 2013; Zhou, Cattley, Cario, Bai, & Burton, 2014), yet, studies on motor functions on adult zebrafish are rare. In prior studies on adult zebrafish, fish activity was investigated through the analysis of time spent swimming (Blazina et al., 2013; Cianca et al., 2013; Spinello et al., 2013), time spent freezing (Cianca et al., 2013; Gerlai, Lee, & Blaser, 2006; Spinello et al., 2013), distance traveled (Gerlai, Ahmad, & Prajapati, 2008; Gerlai et al., 2006), average speed (Ladu et al., 2014; Mathur & Guo, 2011), and number of segments crossed in the tank (Gerlai et al., 2000; Maximino, da Silva, Gouveia, & Herculano, 2011). While these measures are valid indicators of zebrafish activity and have been shown to be affected by ethanol treatment, they should be considered as indirect, rather than direct measures of motor functions.

Acute ethanol exposure has been shown to induce changes in zebrafish locomotion, with higher locomotor activity at low concentrations and, vice versa, lower activity at high concentrations (Blazina et al., 2013; Cianca et al., 2013; Gerlai et al., 2000; Ladu et al., 2014; Maximino et al., 2011; Spinello et al., 2013). The time spent swimming by fish in a spinning tank has been found to decrease at low ethanol concentrations (0.25%) (Blazina et al., 2013), while similar doses have been observed to play a secondary role in preference tests conducted in placid water (Cianca et al., 2013; Spinello et al., 2013). Ethanol administration also influences the time spent freezing by fish in placid water (Cianca et al., 2013; Spinello et al., 2013), whereby subjects treated at high ethanol concentrations (1.00%) exhibit enhanced freezing response. Additionally, for experiments in placid water, both the number of crossings between different segments of the experimental tank (Gerlai et al., 2000; Maximino et al., 2011) and the average speed (Ladu et al., 2014) were found to display a U–shaped curve, with

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each of these quantities being highest at low ethanol concentrations. While studies on zebrafish tail-beat motion are scarce, experimental observations on several aquatic species have demonstrated a positive correlation between swimming speed, or distance traveled, and both the tail-beat frequency and tail-beat amplitude across a wide range of physical scales, from larvae to whales (Bainbridge, 1958; Gazzola, Argentina, & Mahadevan, 2014).

Through a novel automated tracking algorithm capable of reconstructing fish body shape, we sought to elucidate the effects of acute ethanol treatment on the tail-beat amplitude and frequency of adult zebrafish. We studied zebrafish locomotion in placid water for different ethanol treatment levels (0.00%, 0.25%, 0.50%, and 1.00%), using a high-resolution camera. Based on previous observations on the effect of ethanol on speed and the dependence of the speed on the tail-beat motion, we predicted that the tail-beat frequency and amplitude of zebrafish would be affected by ethanol concentration. In line with previous studies on the effect of alcohol on zebrafish activity (Echevarria et al., 2011), we expected that either tail-beat frequency, amplitude, or both will increase at low concentrations (0.25%) before registering a decrease at high concentrations (1.00%).

Materials and methods

Ethics statement

The experimental procedure described in this study was approved by the Animal Welfare Oversight Committee of New York University under protocol number 13−1424.

Animals and housing

A total of 48 adult zebrafish (*Danio rerio*) of wild-type variety, approximately 3 cm in body length, were purchased from an on-line aquarium vendor (LiveAquaria.com, Rhinelander, WI, USA) and housed in 37.8-liter (10-gallon) tanks with a maximum stocking density of 0.5 fish per liter. Temperature and pH were maintained at 25 ± 1 °C and 7.2 respectively. The fish were kept on a 14-h light and 10-h dark cycle according to the circadian rhythm of this species (Cahill, 2002; Matthews, Trevarrow, & Matthews, 2002). Experiments were initiated after a 15-day habituation period, and fish were fed daily after the experimental session with commercial flake food between 5:00 and 6:00 PM each day.

Apparatus

The experimental tank measured 30 × 10 × 15 cm (length × width × height). The tank itself was positioned within a larger square tank, 120 cm in length, and was covered with white contact paper to avoid reflections and provide a high-contrast background for visual tracking. A high-resolution camera (Flea®3 USB 3.0, PointGrey Research, Canada) was mounted 20 cm directly above the tank; the height of the camera was adjusted to ensure maximum coverage of the experimental tank. Four fluorescent tubes of 25 W each (All-Glass Aquarium, UK) were mounted on each side of the square tank. The setup was surrounded by dark curtains to visually isolate the apparatus.

Drugs and treatments

The effect of ethanol administration was tested with four conditions corresponding to the following concentrations: 0.00% (control, equivalent to Mwaffo, Butail, di Bernardo, & Porfiri, 2015), 0.25%, 0.50%, and 1.00%. Absolute ethanol (200 proof) used in the experiment was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Each focal fish was transferred with a hand net from the holding tank to a 500-mL glass beaker filled with water from the experimental tank and treated with the corresponding ethanol concentration, 0% (0 mM), 0.25% (43 mM), 0.50% (86 mM), and 1% (171 mM). The ethanol solution in the beaker was changed for each fish, which was kept in the beaker for 1 h before being placed into the experimental tank. We expect these concentrations to be relatively stable during the 1 h of exposure (Irons, MacPhail, Hunter, & Padilla, 2010). After exposure to ethanol in the beaker, the fish was transferred with a hand net to the experiment tank, which had the same ethanol concentration as the beaker. The 1-h exposure time was selected to allow for stabilization of the blood alcohol levels in the fish (Gerlai et al., 2006; Ryback, 1976; Ryback, Percarpio, & Vitale, 1969).

Experimental procedure

The experimental session was performed in October 2014 and consisted of 12 trials ($n = 12$) for each ethanol concentration. Each trial consisted of two 60-s observations in the experimental tank; the first observation was recorded 30 s after the fish was placed in the tank and the second observation 10 min later (Gerlai et al., 2000). Experimental sessions were video-recorded at 60 frames per second at a resolution of 1280 × 1024 pixels. All videos were stored and analyzed offline with a multi-target shape-tracking algorithm developed in-house during prior work by our group (Bartolini, Butail, & Porfiri, 2014).

Behavioral observations

For each trial, we measured the following quantities: time spent freezing, tail-beat frequency (TBF), tail-beat amplitude (TBA), and average speed. The time spent freezing was computed by dividing the videos into 2-s intervals and counting the number of intervals during which the position of the fish, measured using its centroid on the image, did not move beyond a circle of radius of 2 cm (Kopman, Laut, Polverino, & Porfiri, 2013).

The average speed output from the tracking software was estimated as an optimally weighted difference of the fish centroid in successive frames (Bartolini et al., 2014). Fish tail-tip displacement was also estimated by the tracking algorithm (Bartolini et al., 2014), which fitted a parabola on the fish body in each video frame. Fish TBA and TBF were then obtained from the time series of the tail-tip displacement with respect to the fish head. Specifically, TBA and TBF were computed by identifying spikes in amplitude greater than a threshold of 0.6 cm, exemplifying a tail flick in either direction. The minimum threshold value was set in accordance to the average fin length of the adult zebrafish used for this study. A maximum threshold was set at 1.5 cm to avoid false readings from the reconstruction of the tail-tip position. The TBA was obtained by averaging the amplitude of these spikes over the whole length of the time series, instead of a selected time-window, as was done by Thomas and Janz (2011). The TBF was obtained as the frequency of these spikes in the video frames. If the fish was not moving in an entire video, both TBF and TBA were set to zero.

Statistical analysis

Two-factors ANOVA with replications was performed to study within-group variability, with time-of-observation as the independent variable, and between-group variability across conditions, with ethanol concentration as the independent variable. A Levene's test for homoscedasticity of variances was used to evaluate whether the different ethanol concentrations elicited equally variable or differentially variable inter-individual tail-beat motion (TBA and