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Influences of acute ethanol exposure on locomotor activities of zebrafish larvae under different illumination

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ABSTRACT

Larval zebrafish present unique opportunities to study the behavioral responses of a model organism to environmental challenges during early developmental stages. The purpose of the current study was to investigate the locomotor activities of AB strain zebrafish larvae at 5 and 7 days post-fertilization (dpf) in response to light changes under the influence of ethanol, and to explore potential neurological mechanisms that are involved in ethanol intoxication. AB strain zebrafish larvae at both 5 and 7 dpf were treated with ethanol at 0% (control), 0.1%, 0.25%, 0.5%, 1%, and 2% (v/v%). The locomotor activities of the larvae during alternating light–dark challenges, as well as the locomotor responses immediately following the light transitions, were investigated. The levels of various neurotransmitters were also measured in selected ethanol-treated groups. The larvae at 5 and 7 dpf demonstrated similar patterns of locomotor responses to ethanol treatment. Ethanol treatment at 1% increased the swimming distances of the zebrafish larvae in the dark periods, but had no effect on the swimming distances in the light periods. In contrast, ethanol treatment at 2% increased the swimming distances in the light periods, but did not potentiate the swimming activity in the dark periods, compared to controls. Differences in the levels of neurotransmitters that are involved in norepinephrine, dopamine, and serotonin pathways were also observed in groups with different ethanol treatments. These results indicated the behavioral studies concerning the ethanol effects on locomotor activities of zebrafish larvae could be carried out as early as 5 dpf. The 1% and 2% ethanol-treated zebrafish larvae modeled ethanol effects at different intoxication states, and the differences in neurotransmitter levels suggested the involvement of various neurotransmitter pathways in different ethanol intoxication states.

Introduction

Ethanol is a small molecule that is soluble in both aqueous and lipid environments, which render it permeable to biological membranes, including the blood–brain barrier. The behavioral manifestations of acute ethanol administration in human include psycho-stimulation and euphoria at low and moderate doses, sedation at heavy doses, and death in extreme cases. It has been reported that ethanol has extensive influences on the functions of the central nervous system, ranging from motor ability, perception, and higher cognitive functions, such as learning and memory (Ewing, Mills, Bisgrove, & McManus, 1984; White, Matthews, & Best, 2000).

The zebrafish is an aquatic vertebrate model organism. Although the nervous system of zebrafish is much simpler than that of the mammals, it possesses similar molecular pathways that are involved in the etiology of neurological disorders (Kily et al., 2008; Lockwood, Bjerke, Kobayashi, & Guo, 2004). Therefore, besides its popularity in genetic and developmental studies (Ackermann & Paw, 2003; Udvadia & Linney, 2003), the zebrafish has also been widely employed in neuro-pharmacological studies in order to elucidate the effects of neuroactive drugs on the nervous system (Ellis & Soanes, 2012; Kokel et al., 2010; van der Ven et al., 2005).

It has been reported that, as early as 4–5 days post-fertilization (dpf), zebrafish larvae develop the ability to swim, and exhibit a broad spectrum of behaviors, such as hunting, avoidance, scototaxis, and thigmotaxis (Colwill & Creton, 2011a, b; Schnörr, Steenbergen, Richardson, & Champagne, 2012). Therefore, zebrafish larvae present unique opportunities to study the neurological activities of pharmaceuticals at very early developmental stages. Due to the small sizes of zebrafish larvae, larval behavior studies are

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usually carried out in multi-well plate formats; thus, locomotor activity has been extensively studied as the most straightforward and applicable parameter in investigations on the behavioral influences of environmental factors and neuroactive drugs.

Zebrafish larvae demonstrate behavioral responses to acute ethanol treatment that are similar to rodents. Locomotor activities of zebrafish larvae displayed inverted U-shaped responses under the influence of ethanol. Increased locomotor activities, as measured by swimming speed or swimming distances, were observed in zebrafish larvae treated with ethanol at low concentrations, whereas, further increases in ethanol concentrations resulted in decreases in locomotor activities of the larvae (Ikeda et al., 2013; Lockwood et al., 2004; Puttonen, Sundvik, Rozov, Chen, & Panula, 2013). Mice receiving acute ethanol treatment at various doses demonstrated similar characteristic inverted U-shaped locomotor responses as observed in zebrafish. Low doses of ethanol treatment resulted in increases in locomotor activities, whereas high doses of ethanol treatment exerted inhibitory effects on locomotor activities (Aragon, Pesold, & Amit, 1992; Correa, Miquel, & Aragon, 2000; Phillips, Huson, Gwiazdon, Burkhart-Kasch, & Shen, 1995; Viana, Almeida-Santos, Aguiar, & Moreira, 2013).

Transition in illumination from light to dark usually evokes an initial startle response in zebrafish larvae, followed by increased locomotor activities during the dark phase compared with the precedent light phase (Emran, Rihel, & Dowling, 2008; MacPhail et al., 2009). Acute ethanol treatments were reported to modulate the responses of zebrafish larvae to light changes (Irons, MacPhail, Hunter, & Padilla, 2010; MacPhail et al., 2009). Wild-type zebrafish larvae with unspecified genetic background at 6 dpf demonstrated increased locomotor activities during both the light and the dark phase when treated with 1% and 2% ethanol compared with the control group without ethanol treatment. Ethanol treatment at 4% resulted in complete inhibition of the locomotor activities of the zebrafish larvae, as well as the responses to light changes (Irons et al., 2010; MacPhail et al., 2009). In a different study using AB strain wild-type zebrafish larvae at 6 dpf, ethanol treatment at 1% resulted in increased locomotor activities during both light and dark conditions, whereas, across all illumination conditions, the larvae receiving 2% ethanol treatment demonstrated constant levels of locomotor activities that were comparable to the control group during the light condition (de Esch et al., 2012).

In order to fully utilize the advantages of zebrafish larvae, behavior studies with zebrafish larvae are usually carried out between 5 and 7 dpf. AB is a well-characterized inbred wild-type zebrafish strain with a well-defined genetic background, which is widely employed to investigate the behavioral impacts of various neuroactive drugs. However, the effects of ethanol treatment on the locomotor activities of AB strain zebrafish larvae at 5–7 dpf have not been extensively examined. Therefore, the current study was designed to establish the profile of the locomotor activities of AB strain zebrafish larvae at both 5 and 7 dpf under the influence of ethanol in different illumination conditions, and to explore the possibility of using zebrafish larvae as model organisms to study the neural activities of ethanol and the related molecular mechanisms. The changes in neurotransmitter levels in response to ethanol treatments were also examined to shed light on the potential neurological mechanisms that are involved in ethanol intoxication.

Experimental procedures

Zebrafish husbandry

AB strain wild-type zebrafish were maintained at 28.5 °C according to standard protocols (Westerfield, 1995). Fish were kept on a 14-h light: 10-h dark cycle (lights on at 8:00 AM, lights off at 10:00 PM). Eggs were obtained by natural spawning, and were raised in groups of 50 in an incubator at 28.5 °C from birth to 7 dpf, which was staged according to a previously published method (Kimmel, Ballard, Kimmel, Ullmann, & Schilling, 1995). Eggs and larvae were kept under the same lighting schedule as adult zebrafish. All animal experimental procedures complied with local and international regulations. All protocols were approved by the institutional animal care committee.

Drugs

Ethanol (10009259, Sinopharm Chemical Reagent) working solutions were freshly made by serial dilutions to appropriate concentrations with the zebrafish system water before experiments.

Behavior tests

All behavior tests were performed in a room with an ambient temperature of 28.5 °C. The room was humidified to minimize the evaporation of the water in the testing wells. To ensure adequate swimming spaces for zebrafish larvae, behavior tests were carried out in 24-well plates. The inner diameter of each well was 18 mm, which is about 6 times the body length of a 7 dpf zebrafish larva.

Behavior tests were carried out with the zebrafish larvae at both 5 dpf and 7 dpf. The larvae were obtained from group mating, and were randomly assigned to the 5 dpf group and the 7 dpf group. Since the replicate 24-well dilution series experiments were performed on 2 separate days, 2 batches of group mating were carried out. On each testing day, 4 plates were tested consecutively. All the experiments were performed between 10:00 AM and 6:00 PM. The experiments were arranged in a way that all concentration groups were equally presented in each 24-well plate to avoid any inter-treatment variations due to differences in experiment timing during the day.

The zebrafish larvae were carefully transferred to a 24-well plate with one single larva in each well. Excess fluid was removed, and 500 μL of fresh zebrafish system water was loaded into each well immediately. Subsequently, 500 μL of ethanol working solution was quickly added into the wells; therefore, each well contained 1 mL liquid. The final ethanol concentrations tested were 0% (control), 0.1%, 0.25%, 0.5%, 1%, and 2% (v/v%). The substantial volume of the ethanol working solution ensured a good mixture of the liquid in the wells. In addition, the relatively small size of the well avoided the occurrence of strong currents that might agitate the larvae.

The plate was then placed into a Zebrabox apparatus (ViewPoint Life Sciences) to video record the zebrafish larvae activities. The zebrafish larvae were first given a 50 min acclimation period with illumination at 110 Lx. Then three 15-min cycles (10 min illumination at 110 Lx followed by 5 min dark, i.e., illumination off) were delivered to examine the responses of the zebrafish larvae to changes in lighting conditions under the influence of ethanol. Therefore, each experiment session lasted 95 min, including the acclimation period, and ethanol was presented during the whole 95 min experiment session.

The quantification of zebrafish larvae locomotion activities was achieved using the tracking mode of Zebralab software (ViewPoint Life Sciences) with recorded videos. The videos of zebrafish larvae were taken at the rate of 25 fps, and were pooled into 1 min time bins. The distance moved by the larvae in the whole well was acquired for the analysis of locomotor activities.

Analysis of neurotransmitters and systemic ethanol level

A separate set of zebrafish larvae at 7 dpf were used for analysis. The larvae were treated with 0% (control), 0.5%, 1%, and 2% (v/v%)