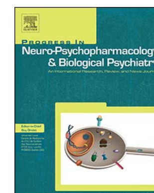
Progress in Neuropsychopharmacology & Biological Psychiatry 79 (2017) 105–111

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

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



Repeated ethanol exposure alters social behavior and oxidative stress parameters of zebrafish

Talise Ellwanger Müller[a,b,*], Stenio Zimermann Nunes[a,b], Ariane Silveira[a,b], Vania Lucia Loro[b], Denis Broock Rosemberg[a,b,c,*]

[a] Laboratory of Experimental Neuropsychobiology, Department of Biochemistry and Molecular Biology, Center of Natural and Exact Sciences, Federal University of Santa Maria, 1000 Roraima Avenue, Santa Maria, RS 97105–900, Brazil

[b] Graduate Program in Biological Sciences: Toxicological Biochemistry, Federal University of Santa Maria, 1000 Roraima Avenue, Santa Maria, RS 97105–900, Brazil

[c] The International Zebrafish Neuroscience Research Consortium (ZNRC), 309 Palmer Court, Slidell, LA 70458, USA

A R T I C L E I N F O

Keywords:
Ethanol
Oxidative stress
Shoaling
Zebrafish
Chronic exposure

A B S T R A C T

Repeated ethanol (EtOH) consumption induces neurological disorders in humans and is considered an important public health problem. The physiological effects of EtOH are dose- and time-dependent, causing relevant changes in the social behavior. In addition, alcohol-induced oxidative stress has been proposed as a key mechanism involved in EtOH neurotoxicity. Here we investigate for the first time whether repeated EtOH exposure (REE) alters the social behavior of zebrafish and influences brain oxidation processes. Animals were exposed to water (control group) or 1% (v/v) EtOH (EtOH group) for 8 consecutive days (20 min per day). EtOH was added directly to the tank water. At day 9, the social behavior and biochemical parameters were assessed. REE increased shoal cohesion by reducing inter-fish and farthest neighbor distances. SOD and CAT activities, as well as NPSH levels decreased in brain tissue. Moreover, REE increased lipid peroxidation suggesting oxidative damage. In summary, changes in oxidation processes may play a role in the CNS effects of EtOH, influencing the social behavior of zebrafish. Furthermore, in a translational neuroscience perspective, our data reinforces the utility of zebrafish to clarify the biochemical and behavioral effects of intermittent EtOH administration.

1. Introduction

Alcohol consumption is associated with a wide spectrum of negative health outcomes including morbidity, disability, and mortality (Global Status Report on Alcohol and Health - World Health Organization, 2014). Alcohol abuse and dependence lead to economic problems due to the costs of healthcare (Sacks et al., 2015) since alcoholic individuals are more susceptible to develop severe neurological disorders (Costardi et al., 2015). Importantly, different neurotransmitters and intricate transduction signaling pathways mediate the psychotropic effects of ethanol (EtOH) (Esel, 2006; Rico et al., 2011a, 2011b).

At a behavioral level, low to moderate EtOH doses induce stimulant effects on behavior, decreasing anxiety and contributing to drug abuse. Conversely, chronic alcohol consumption increases anxiety, leading to deleterious effects on brain functions (Camarini et al., 2010; Gerlai et al., 2000; Rosemberg et al., 2012). Several mechanisms involved in EtOH-mediated neurotoxicity have been proposed and oxidative stress is usually associated with deleterious effects (Augustyniak et al., 2005; Pereira and Andrade, 2015; Sun and Sun, 2001; Sun et al., 2001). Brain EtOH catabolism involves enzyme activities (e.g. catalase, alcohol dehydrogenase, and cytochrome P450) and naturally produces reactive oxygen species (ROS) (e.g. superoxide free radicals, hydrogen peroxide, and hydroxyl radicals) (Haorah et al., 2008; Hipólito et al., 2007; Zakhari, 2006). However, an excessive ROS production may alter the central nervous system (CNS) redox state, impairing DNA, lipid, and protein metabolism (Bondy, 1992; Comporti et al., 2010). Animals chronically intoxicated with EtOH showed significant changes in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) activities (Augustyniak et al., 2005; Hernández et al., 2016; Zimatkin and Buben, 2007). These data clearly suggest an involvement of oxidant processes in the neurobehavioral actions of alcohol.

During the last decades, new experimental models have been validated to assess the effects of EtOH in vertebrates (Kaun et al., 2011; Spanagel, 2010; Tran and Gerlai, 2014). In this context, the investigation of evolutionarily conserved mechanisms is a valuable strategy to understand the basis of alcohol abuse and addiction in translational neuroscience. Zebrafish (*Danio rerio*) is a prominent model organism to

* Corresponding authors at: 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.

E-mail addresses: talise_tm@yahoo.com.br (T.E. Müller), dbrosemberg@gmail.com (D.B. Rosemberg).

http://dx.doi.org/10.1016/j.pnpbp.2017.05.026
Received 30 March 2017; Received in revised form 24 May 2017; Accepted 30 May 2017
Available online 07 June 2017
0278-5846/ © 2017 Elsevier Inc. All rights reserved.

assess the neurochemical and behavioral effects of EtOH (Tran et al., 2016). In addition to the high genome conservation (Howe et al., 2014), this species displays the major neurotransmitter systems that mediate EtOH responses (Chatterjee et al., 2014; Gerlai et al., 2009; Miller et al., 2013). Importantly, EtOH also exerts a biphasic effect on zebrafish behavior by exacerbating vertical activity and aggression at low concentrations, whereas locomotion and social preference decrease at higher concentrations (Fontana et al., 2016; Ladu et al., 2014; Rosemberg et al., 2012).

Regarding the social behavior, adult zebrafish have a natural tendency to form shoals. Shoaling is a highly complex behavior involved in foraging strategies, anti-predatory behaviors, and mating (Buske and Gerlai, 2011; Fernandes et al., 2015; Miller et al., 2013; Miller and Gerlai, 2007; Miller and Gerlai, 2011). Various human brain diseases are linked with disrupted group behavior and chronic alcohol intoxication may influence social behavior components (Gerlai, 2014; Kalueff et al., 2015). In this report, we investigate whether REE alters the social behavior of zebrafish and modulates oxidative stress parameters in the brain.

2. Material and methods

2.1. Animals

All experiments were performed using 64 adult (4–6 months-old) zebrafish (*Danio rerio*). Considering the conflicting data about the sex and EtOH influence on social behavior (Etinger et al., 2009; Kurta and Palestis, 2010; Fernandes et al., 2015), and the random use of male and female fish in different behavioral paradigms with reproducible data (Canzian et al., 2017; Egan et al., 2009; Green et al., 2012; Maximino et al., 2010), zebrafish of mixed genders (50:50 male:female ratio) were used. Short fin wild-type (*WT*) zebrafish were obtained from a local supplier (Hobby Aquários, RS, Brazil) and acclimated in 40-L tanks for two weeks in a maximum density of four fish per liter. Tanks were filled with non-chlorinated water kept under constant mechanical, biological, and chemical filtration at 26 ± 2 °C. The pH and conductivity were monitored and set at 7.0–8.0 and 1500–1600 μS/cm, respectively. Illumination was provided by ceiling-mounted fluorescent light tubes kept on a 14/10 light/dark photoperiod cycle (lights on at 7:00 am). Animals were fed with a commercial flake fish food (Alcon BASIC®, Alcon, Brazil) twice daily. All experiments were performed in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals and the protocols were approved by the Ethics Commission on Animal Use of the Federal University of Santa Maria (process number 026/2014).

2.2. Exposure protocol

REE was performed as described previously (Mathur and Guo, 2011). Initially, zebrafish were kept in housing tanks (25 cm length × 15 cm height × 6 cm width) separated in groups of 4 animals per shoal. Although zebrafish form larger shoals in their natural environment, previous studies showed reproducible data of social behavior using 4-fish shoals (Canzian et al., 2017; Schmidel et al., 2014; Green et al., 2012). Zebrafish shoals (n = 8) were exposed to 1% v/v EtOH for 8 consecutive days (20 min per day). Control fish (n = 8 shoals) were handled in a similar manner, except that no EtOH was added. Later, fish were returned into their housing tanks. We used the intermittent ethanol exposure protocol due to its translational relevance since it closely resembles what human drinkers would experience (Alcohol-Alert, 2001). No physical abnormalities were observed during the exposure period and at day 9, the behavioral and biochemical parameters were evaluated. Fig. 1 shows a schematic representation of the experimental protocol.

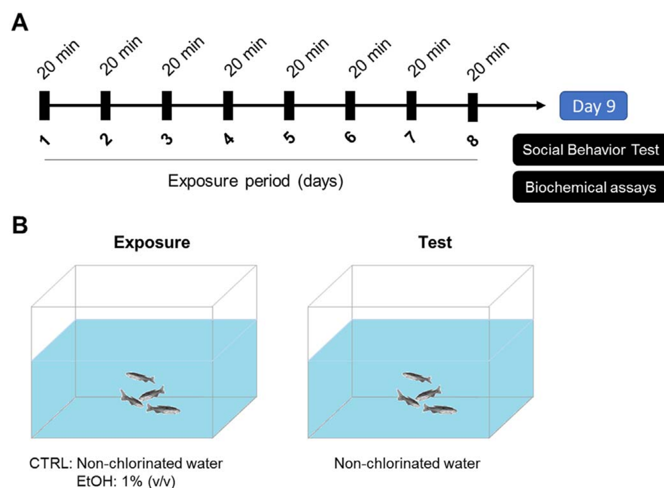


Fig. 1. Schematic representations of the methodological approach used for the evaluation of the social behavior and biochemical parameters in zebrafish using a REE protocol. (A) Experimental design and endpoints measured. (B) Illustration of the zebrafish group and experimental conditions during exposure period and behavioral tests.

2.3. Social behavior task

After the exposure period, each zebrafish group was placed in the test tank (25 cm length × 15 cm height × 6 cm width). Tank dimensions were similar to those used in previous reports that assessed the group behavior of zebrafish (Canzian et al., 2017; Green et al., 2012). Water column was 10 cm depth and the social behavior was recorded during a 6-min period. The videos were further exported to Image J 1.49 software for Windows™ and shoaling was determined using screenshots made every 15 s over the test period (with a total of 25 screenshots per group). 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 mean dispersion (shoal area). The vertical distribution of zebrafish (number of animals in the top area of the tank) was evaluated by manually scoring the number of animals in the upper half of the apparatus every 15 s over the test period. Two trained observers (inter-rater reliability > 0.85) blind to the experimental conditions analyzed the videos and all measures were performed as described previously (Canzian et al., 2017; Schmidel et al., 2014). All behavioral analyses were performed using eight independent treatments per shoal of 4 fish (n = 8) for both control and EtOH groups.

2.4. Biochemical assays

2.4.1. Tissue preparation

At day 9, zebrafish were gently netted from their home tanks and rapidly euthanized by decapitation. For each independent preparation, four brains were pooled and homogenized on ice in 1 mL Tris HCl buffer (50 mM pH 7.4). Samples were further centrifuged at 3000 $\times g$ for 10 min at 4 °C and the supernatants were kept in microtubes at − 80 °C for posteriors assays.

2.4.2. Quantification of SOD activity

SOD activity was assessed by testing the inhibition of the radical superoxide reaction in the presence of adrenalin (Misra and Fridovich, 1972). The reaction was quantified by monitoring adrenochrome formation at 480 nm in a medium containing glycine-NaOH buffer (50 mM, pH 10), adrenaline (1 mM) and homogenate (20–30 μg protein) (Müller et al., 2017; Nunes et al., 2016). A unit of SOD was defined as the amount of enzyme that inhibits 50% of adrenaline oxidation rate. SOD activity was determined in a microplate reader and expressed as U

Download English Version:

https://daneshyari.com/en/article/5557911

Download Persian Version:

https://daneshyari.com/article/5557911

Daneshyari.com