



## A dose for the wiser is enough: The alcohol benefits for associative learning in zebrafish



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### ABSTRACT

This study aimed to test seeking behavior caused by alcohol and the drug effects on learning in the zebrafish, *Danio rerio*. Three treatments were conducted: acute, chronic and withdrawal, using 0.10%, 0.25%, and 1.00% alcohol and control (0.00%) (vol/vol.%). For the drug seeking behavior, we used a place preference paradigm (shuttle box tank) before and after alcohol exposure in acute (single exposure) and chronic (7 days) treatments. We observed a change in the basal preference due to the association with alcohol only for 0.25% and 1.00% doses in both acute and chronic offering, indicating an alcohol-seeking behavior after the drug exposure. For the learning task, two treatments were tested: chronic alcohol exposure (26 days including the learning period) and alcohol withdrawal (15 days of alcohol exposure before the learning period). During the learning period, fish received light stimulus followed by food in a pre-defined area of the tank for 8 consecutive days. The low dose group (0.10%) learned the task by the 3rd day both in chronic and withdrawal treatments. The higher doses (0.25% and 1.00%) caused a learning impairment in the chronic treatment group, while fish from the alcohol withdrawal treatment displayed learning on the final testing day. Therefore, we suggest that high alcohol doses impair learning and cause drug seeking behavior, even after drug exposure cessation, while low doses positively affect learning and do not cause seeking behavior. Given our results we propose that the zebrafish is a promising model for identifying active compounds, antibodies or genes which modulate the alcohol dual effects: learning improvement and reinforcing behavior.

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### 1. Introduction

Learning and memory are processes affected by physiological and neural changes due to psychoactive drug use (Gould, 2010). Among these, alcohol has been widely investigated because of the allowed use (licit drug) and the enormous impact on the health of society (Lima, 2003; OMS, 2003). The neurological and psychological effects in short and long term uses show a dual effect: moderate doses cause stimulating and anxiolytic effects, which are factors that promote addiction (Carlson, 2001), while higher doses lead to motor control loss,

disorientation and sedation — part of the depressive effect (Gerlai, 2013; Quoilin et al., 2013; Roseribloom et al., 2004).

The addictive component of alcohol use involves a compulsive drug seeking behavior even after long abstinence (Brennan et al., 2011), which is associated with the increased dopaminergic transmission in the mesolimbic system (Rink and Wullimann, 2002). The alcoholic develops necessity for alcohol intake and also becomes tolerant to the drug. This response seems to occur due to neuroadaptation at the brain signaling level or altered alcohol metabolism (Tran and Gerlai, 2013). The functional tolerance that can be observed behaviorally (Arias et al., 2012; Gerlai et al., 2006) may facilitate the consumption of increasing amounts of alcohol, which is well known to provoke high damage to cognitive processes (Beveridge et al., 2013; Crews and Nixon, 2009; Obernier et al., 2002). However, the genetic and neuroethological basis of the seeking behavior and addiction needs to be deeply comprehended in order to indicate pharmacological and psychological therapies for the drug addiction treatment.

Several studies point out that there is lack of research on alcohol dose–effect and how it acts on the brain (Fuller and Hiller-Sturnhöfel, 1999; Gerlai et al., 2009; Vengeliene et al., 2008). Recent studies suggest negative (Cruz et al., 2009; Kaley and During, 2007; Scheffer et al., 2010;

*Abbreviations:* CPP, Conditioned place preference; RM Anova, Repeated Measures Analysis of Variance; CNS, Central Nervous System; NMDA, N-Methyl-D-Aspartate; pCREB, phosphorylated cAMP response element-binding protein; CNPq, National Counsel of Technological and Scientific Development; CAPES, Coordenação de Aperfeiçoamento de Pessoas de Nível Superior.

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Tuck and Jackson, 1991) and positive (Ruitenberget al., 2002; Weyerer et al., 2011) effects of alcohol on brain functioning, both of which are dependent on dosage. However, there is still little research done on alcohol-seeking behavior and cognitive effects with low doses.

Therefore, we aimed to use the zebrafish to study 1) the drug seeking behavior caused by different alcohol doses in short and long term uses and 2) the effects of different alcohol doses on a learning task with a cognitive element (cognition test). Behavioral states of zebrafish after exposure to alcohol have been characterized (Gerlai, 2013; Gerlai et al., 2000), which suggest that the zebrafish is a good model for studying the biological effects of alcohol, addiction and withdrawal syndrome (Cachat et al., 2010; Darland and Dowling, 2001; Lau et al., 2011; Mathur et al., 2011; Ninkovic and Bally-Cuif, 2006). Moreover, the central nervous system of the zebrafish is similar to mammals, making our results translational to human diseases (Klee et al., 2012; Kolb and Whishaw, 1998).

## 2. Methods

Adult zebrafish, *Danio rerio* (Hamilton, 1822) acquired from a local ornamental fish farm (Natal, RN) were held in stock tanks (1 fish/L) with aerated and filtered water. Four 40 L tanks formed a stock unit in a closed recirculation system with mechanical, biological and chemical filtration and UV disinfection, which maintained water at  $28 \pm 1$  °C, pH 7.2 and low levels of ammonia and nitrite. Illumination was set on a 12/12-light/dark cycle. Fish were fed twice a day ad libitum with commercial food (38% protein, 4% lipid, Nutricom Pet). All animal procedures were performed with the permission of the Ethical Committee for Animal Use of the Universidade Federal do Rio Grande do Norte (CEUA 024/2012).

### 2.1. Drug seeking behavior test

The experimental strategy was the evaluation of the seeking behavior caused by different alcohol doses in acute and chronic treatment on a conditioned place preference paradigm (CPP), adapted from the procedures used by Brennan et al. (2011) and Mathur et al. (2011).

The testing apparatus used was a 15 L aquarium divided in half with an opaque glass divider (shuttle box,  $40 \times 25 \times 20$  cm). Each bottom side of the tank had different visual cues (one totally white versus the other in a black and white grid,  $2 \times 2$  cm; Fig. 1). Lateral walls of the tank were all covered in white. Basal preference was determined for each fish by individually introducing them in the shuttle box and after a 2 min acclimation, recording (Sony Digital Video Camera Recorder; DCR-SX45) behavior from above for 5 min. The videos were analyzed using ANY-maze™ Video Tracking System, which registered the time spent in each side of the shuttle box. The side where fish spent more than 60% of the total time was considered the preferred place. After

the basal preference test, fish were netted to individual aquarium (700 mL) until the next day.

On the day after basal preference determination, fish were submitted to conditioning. Each fish was restricted first to the least preferred side for 20 min in the presence of alcohol. For that, we used a smaller aquarium ( $20 \times 20 \times 10$  cm, 2 L) placed inside the shuttle box, allowing fish to see the bottom. Then fish was restricted to the preferred side for 20 min in freshwater. Regardless of the basal preference, fish were always placed in alcohol in the least preferred side first and then placed in freshwater in the preferred side. This procedure ensured that the fish were exposed to alcohol only in the least preferred location. Following this procedure, each fish was netted to its individual aquarium.

Two alcohol treatments were tested: acute and chronic. Alcohol concentrations of 0.10%, 0.25% and 1.00% were achieved by diluting alcohol (Anhydrous Ethyl Alcohol; 100%) into water. The control group was kept always in 0.00% alcohol (acute  $n = 8$ , chronic  $n = 8$ ). Animals submitted to acute treatment received alcohol only the day after basal preference determination (0.10%  $n = 10$ , 0.25%  $n = 11$ , 1.00%  $n = 13$ ). Fish from chronic treatment were submitted to alcohol for 7 consecutive days, always in the least preferred location (0.10%  $n = 8$ , 0.25%  $n = 9$ , 1.00%  $n = 8$ ).

To define the reinforcing effects of alcohol, the place preference of each fish was tested either the following 5 days after single exposure (acute effects) or after 7 days of conditioning (chronic effects). Animals were recorded for 5 min (as for the basal preference test) to observe possible changes in preference. All fish tracking was performed using the ANY-maze™ Video Tracking System.

The percentages of time spent in each side of the shuttle box were compared between basal preference and 5 days after alcohol submission. We compared acute and chronic treatments using repeated-measures Anova (Analysis of Variance), since data passed the normality and equal variance tests. We used the Student–Newman–Keuls post-hoc test. A probability level of  $p < 0.05$  was used as an index of statistical significance.

### 2.2. Cognition test

In this test we evaluated the effect of different alcohol doses on associative learning using a cognitive element. There were two experimental groups tested: 1) chronic treatment: exposed to alcohol for 18 days + 8 days during the learning test, and 2) withdrawal treatment: exposed to alcohol for 15 days + 3 days of acclimation without alcohol and 8 days of learning test without alcohol. Alcohol doses were 0.10%, 0.25% and 1.00% alcohol and the control group (dose 0.00%). Before the learning test, fish were submerged in alcohol for a period of 20 min each day in a 15 L aquarium ( $40 \times 20 \times 25$  cm) and then returned to their home tanks (freshwater) for the remainder of the time until the next alcohol exposure. Fish were submitted to alcohol

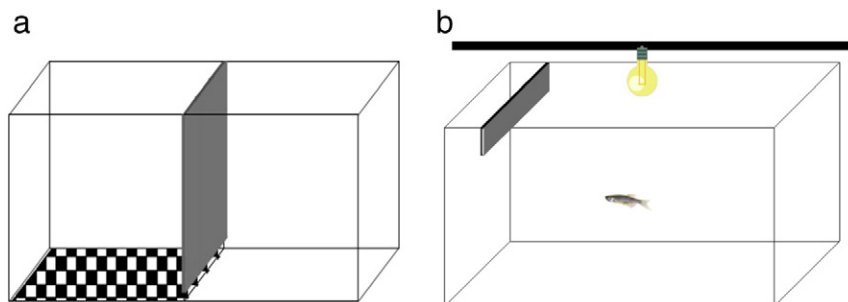


Fig. 1. Schematic draw of a) the shuttle box used for the conditioned place preference test, where all sides were opaque white and bottom was half white half black and white grid with a 2 cm passage between sides, and b) the conditioned learning task aquarium, with the feeding area at the upper left region and the lamp (unconditioned stimulus) above.

in groups of 12. The control group was moved to another aquarium (freshwater) during the same period of alcoholization.

Fish from the chronic treatment were submitted to the drug during the entire experimental period, including the 3 acclimation days and the learning test. For that, a small aquarium (20 × 20 × 10 cm) containing 2 L of alcohol solution was positioned beside each conditioning test aquarium. Alcohol solution was prepared everyday and alcohol submission occurred in the evening. For the control group we used freshwater. This procedure was not done for the withdrawal treatment group.

The conditioning test evaluated zebrafish performance by associating an unconditioned stimulus (food) to a conditioned stimulus (light). For the test, fish were chemically, acoustically and visually isolated from each other in glass aquaria (40 × 25 × 20 cm, 15 L), where there was a small opaque divider at the upper left region delimitating the feeding area (25 × 2 cm) and a lamp on the central area of the aquarium (Fig. 1b). Illumination (910 ± 2 lx) and feeding were used as conditioned and unconditioned stimuli, respectively. Temperature and photoperiod were set the same as in holding conditions; ambient light was 47.5 ± 0.31 lx.

An opaque curtain was placed between the experimenter and the aquarium so that the light could be adjusted and fish could be fed without the experimenter being visible to the fish. This eliminated the possibility of the fish associating the researcher to the unconditioned stimulus. Food was offered once a day using a manual feeder (a small recipient connected to a long tube), always delivered at the feeding area just after the light stimulus (4 pellets of food; the same food used for stock fish). Fish were fed only during the experimental period (once a day) in order to maintain motivation for learning (fish were never food deprived). The total number of fish used for the tests was 40 for the chronic treatment (10 fish for each dose) and 48 for the withdrawal group (12 for each dose).

Fish were kept for 3 days in these conditions for acclimation; no light stimulus was given during this period. After that, fish behavior was recorded daily using a digital video camera during a 2 min and 10 s period for 8 consecutive days. The records included 1 min before conditioned stimulus and 1 min after the end of the stimulus. Light (conditioned stimulus) was offered for 10 s, followed by food delivery. This methodology was previously used by Luchiani and Chacon (2013).

Fish activity in the aquarium and the distance from the feeding area were analyzed. Activity of the fish was measured by the dispersion area 1 min before and 1 min after the light stimulus. The distance of the fish to the feeding area was measured every 10 s. We used this data to calculate the mean distances before and after the light stimulus. The difference between the distance from the feeding area before and after the conditioned stimulus, was used to calculate the approaching index [AI = (distance from the feeding area after light stimulus) – (distance from the feeding area before light stimulus)]. To validate the index used, we compared the position of the fish prior to the light stimulus on each occasion. We were able to infer learning using this index: a large amount of negative values indicated that the fish approached the feeding area more often. The mean approaching index from the 8-recorded days of conditioning was analyzed during a 2-day period block.

The Friedman test was used to compare the position of the fish before the light stimulus, aiming to validate the approaching index. Repeated-measures Anova were used to analyze dispersion and approaching index of each treatment. The post hoc test used was Student–Newman–Keuls. A probability level of  $p < 0.05$  was used as an index of statistical significance.

### 3. Results

#### 3.1. Drug seeking behavior test

We observed that 13 animals from the acute treatment showed basal preference for the white side of the shuttle box, while 22 animals preferred the grid side.

The control group of the acute treatment (shuttle box with water only) did not show significant change in place preference (RM Anova,  $F = 0.87$   $p = 0.51$ ; Fig. 2a). Animals subjected to acute doses of alcohol showed variation in the preference conditioned response in a dose dependent way (Fig. 2). Animals that were subjected to 0.1% alcohol did not change basal preference, while maintaining the highest visitation to the same environment preferred on the first day (RM Anova,  $F = 1.99$   $p = 0.10$ ; Fig. 2b). However, animals receiving acute alcohol in concentrations of 0.25 and 1.00% changed side preference to where they experienced the drug. The animals which experienced 0.25% alcohol remained longer in the initially not preferred location on days 2, 4 and 5 (RM Anova,  $F = 4.05$   $p = 0.005$ ; Fig. 2c); and the animals which consumed 1.00% alcohol altered their environmental preference completely, remaining during the 5 days post-alcohol in the compartment not initially preferred (RM Anova,  $F = 2.73$   $p = 0.02$ ; Fig. 2d).

The basal place preference of the animal submitted to the chronic treatment was: 14 fish preferred the white side of the shuttle box and 11 preferred the grid side. The control group of the chronic treatment did not change place preference throughout the days (RM Anova,  $F = 1.70$   $p = 0.15$ ; Fig. 3a). For the other groups under chronic alcohol exposure, the overall behavior resembled those under acute treatment (Fig. 3). The 0.10% alcohol group did not alter basal preference by alcohol exposure, keeping the preference shown on the first day (RM Anova,  $F = 0.56$   $p = 0.19$ ; Fig. 3b). Once again only the groups subjected to 0.25 and 1.00% alcohol showed significant behavioral change. The fish subjected to 0.25% alcohol for 7 days stayed longer on the side which wasn't preferred on days 1, 2, 3 and 5 post-alcohol exposure (RM Anova,  $F = 2.35$   $p = 0.048$ ; Fig. 3c); and the animals submitted to the dose of 1.00% chronically, stayed longer in the alcohol environment (not preferred side) on days 2, 4 and 5 (RM Anova  $F = 3.49$   $p = 0.01$ ; Fig. 3d).

#### 3.2. Cognition test

##### 3.2.1. Chronic treatment

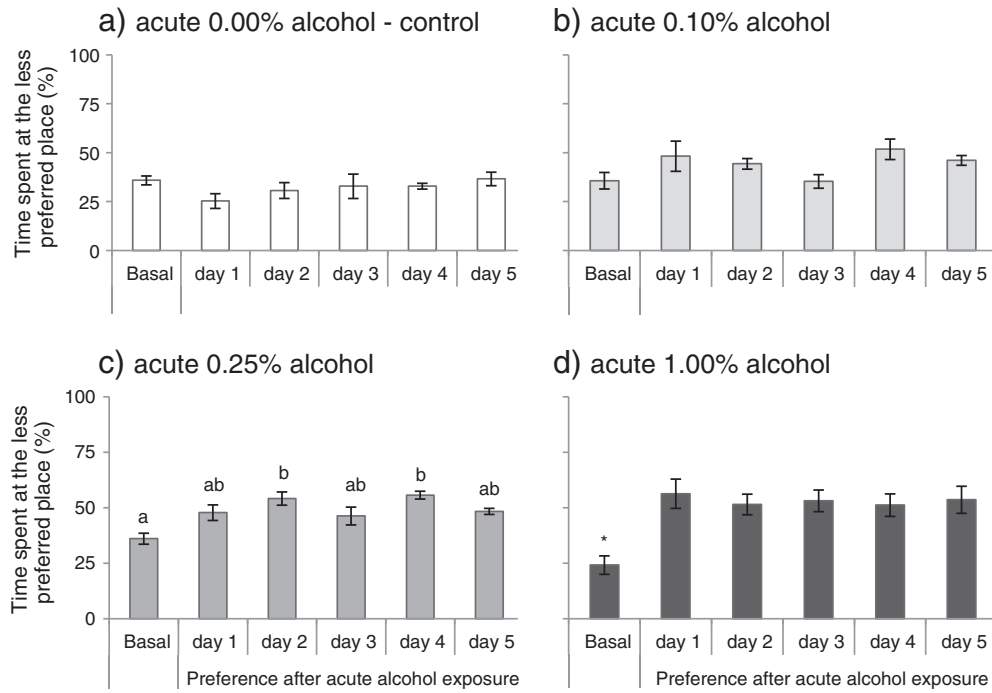
The dispersion of the fish did not vary over the days (RM Anova 0.00%:  $F = 0.84$   $p = 0.48$ ; 0.10%:  $F = 1.26$   $p = 0.30$ ; 0.25%:  $F = 0.24$   $p = 0.87$ ; 1.00%:  $F = 2.18$   $p = 0.10$ ). However, there was a difference detected between groups (index validation) of fish approaching the feeding site before light stimulus. That is, animals in the 0.25% group were significantly closer to the feeding site than animals from the 0.00%, 0.10% and 1.00% groups (Anova,  $F = 3.46$   $p = 0.02$ ).

Regarding the approaching index, it decreased over the days for the control group and 0.10% alcohol (Fig. 4a and b), but did not change for groups subjected to doses of 0.25% and 1.00% alcohol (Fig. 4c and d). The control group significantly approached the feeding area on the 3rd block of days (days 5 and 6; RM Anova,  $F = 3.495$   $p = 0.021$ , Fig. 4a). The 0.10% alcohol group significantly approached the feeding site on the 2nd block of days (days 3 and 4; RM Anova,  $F = 2.942$   $p = 0.041$ , Fig. 4b). Meanwhile we found no significant results for the 0.25% alcohol group (RM Anova  $F = 0.973$   $p = 0.412$ , Fig. 4c) nor for the 1.00% alcohol group (RM Anova,  $F = 0.807$   $p = 0.496$ , Fig. 4d).

##### 3.2.2. Withdrawal treatment

Only the controlled group (dose 0.00%) varied in dispersion over the days (0.00%: RM Anova,  $F = 5.115$   $p = 0.003$ ; 0.10%: RM Anova,  $F = 1.295$   $p = 0.284$ ; 0.25%: Friedman,  $\chi^2 = 1.850$   $p = 0.604$ ; 1.00%: RM Anova,  $F = 1.93$   $p = 0.134$ ). The position of the fish before the light signal did not differ between withdrawal groups from 0.00%, 0.10%, 0.25% and 1.00% alcohol (index validation; Kruskal–Wallis,  $H = 1.76$   $p = 0.41$ ).

There was an increasing reduction in the approaching index over the test days for all experimental groups (Fig. 5). The control group showed significant approach to the feeding area by the 3rd block of days (days 5 and 6) and maintained the response until the end of the test (RM Anova,  $F = 9.46$   $p < 0.001$ , Fig. 5a). The group in withdrawal from 0.10% alcohol

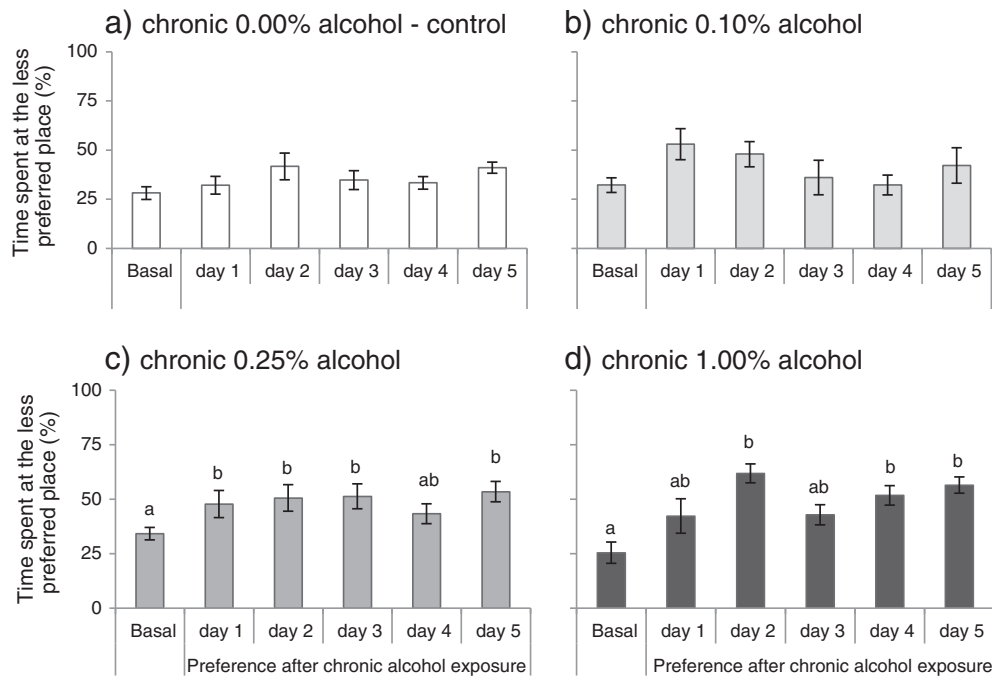


**Fig. 2.** Conditioned place preference induced by acute alcohol exposure. Bars indicate mean  $\pm$  SEM time percentage in the less preferred place in a shuttle box. Basal is the initial preference test and days 1 to 5 are preference after alcohol exposure (0.00% n = 8, 0.10% n = 8, 0.25% n = 9 and 1.00% n = 8). Same letters mean non-significance and different letters /\* indicate significance value (RM Anova,  $p < 0.05$ ).

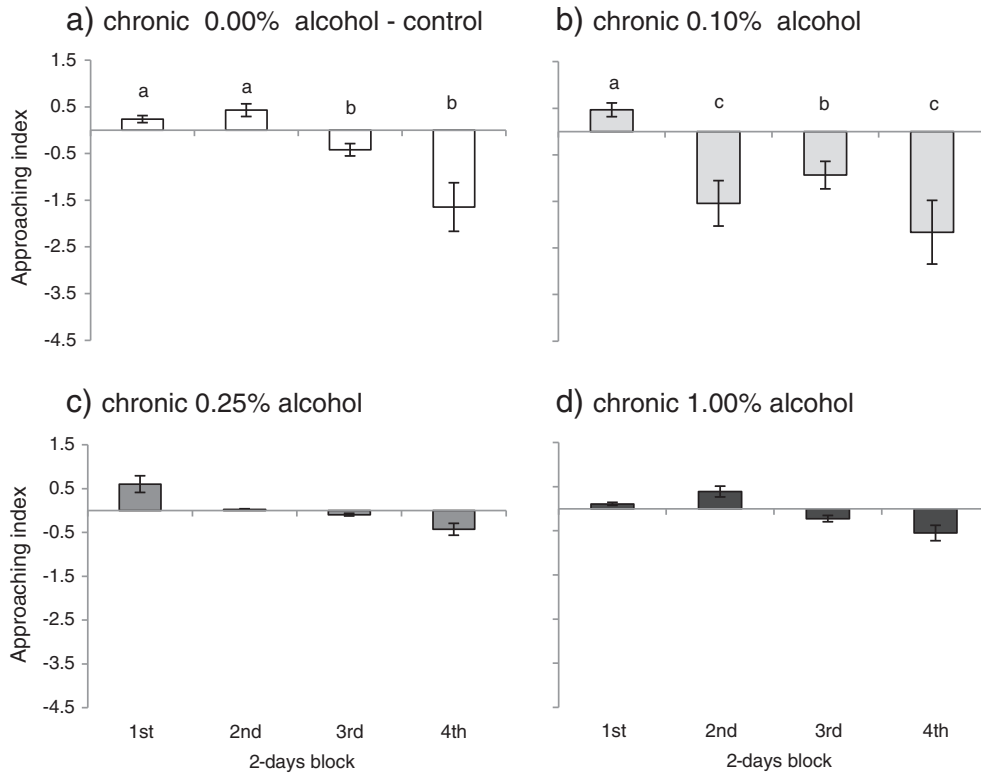
showed approach towards the feeding area after the light signal in the second block of days (days 3 and 4) and then increased this response by the last block of days (days 7 and 8) (RM Anova,  $F = 25.25$   $p < 0.001$ , Fig. 5b). The experimental groups that were in withdrawal from 0.25 and 1.00% alcohol showed significant approach by the last block of days (days 7 and 8) (RM Anova 0.25%:  $F = 16.78$   $p < 0.001$ ; and 1%:  $F = 17.09$   $p < 0.001$ , Fig. 5c and d).

**4. Discussion**

We observed that alcohol alters zebrafish's conditioned learning and promotes seeking behavior in a dose-dependent manner. Low doses of the drug (0.10%) still enabled a learning response and generated no seeking behavior, while higher doses (0.25% and 1.00%) impaired the associative performance and induced search for the drug.



**Fig. 3.** Conditioned place preference induced by chronic alcohol exposure. Bars indicate mean  $\pm$  SEM time percentage in the less preferred place in a shuttle box. Basal is the initial preference test and days 1 to 5 are preference after alcohol exposure for 7 days (0.00% n = 8, 0.10% n = 10, 0.25% n = 10 and 1.00% n = 10). Different letters mean significance value while at least one same letters indicate non-statistical significance (RM Anova,  $p < 0.05$ ).

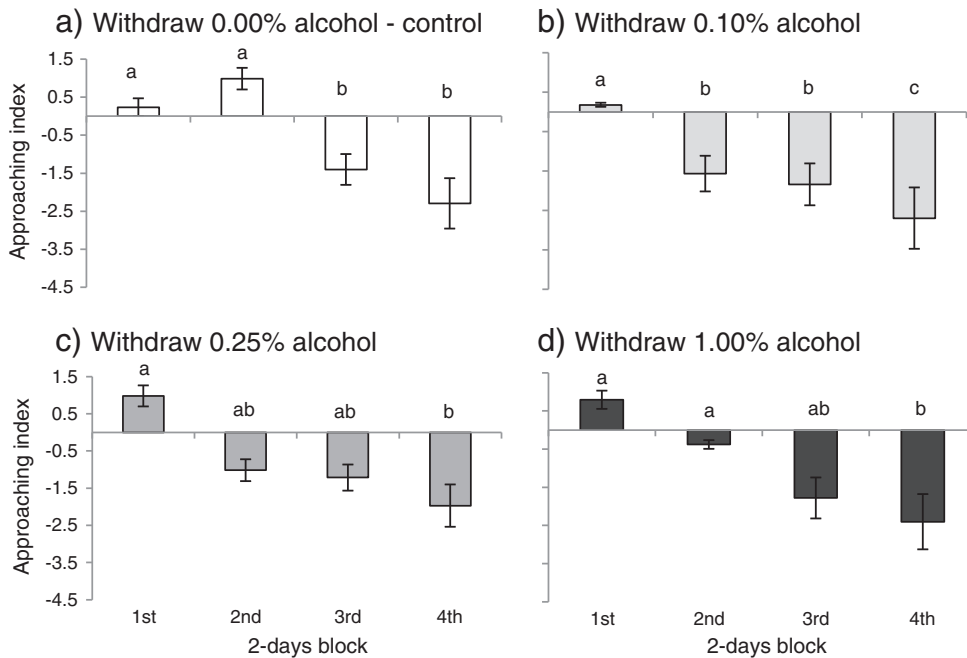


**Fig. 4.** Approaching index of the feeding area ± SEM of the zebrafish during 8 days of the conditioning test using food as reinforcement. Each group was exposed to chronic alcohol treatment for 26 days (n = 10 for each group). Different letters indicate statistical differences between different blocks of days (RM Anova, p < 0.05).

Our findings confirm previous studies where zebrafish (absence of alcohol) showed associative learning (Al-Imari and Gerlai, 2008; Braubach et al., 2009; Gómez-Laplaza and Gerlai, 2010; Karnik and Gerlai, 2012; Luchiani and Chacon, 2013). We also show here that animals treated with low alcohol doses (0.10%) were able to learn to associate stimuli at least 2 days in advance of the control group. On the other hand, 0.25% and 1.00% chronic alcohol treatment inhibited learning

behavior. However, with the cessation of alcohol exposure, fish still learned the task (but at a later time), indicating some kind of recovery from the harmful effects of alcohol. Moreover, we found that only 0.25% and 1.00%, both in acute or chronic use, generate alcohol seeking behavior.

It is well established that alcohol affects almost all zebrafish behavior (Gerlai, 2013; Gerlai et al., 2000). For instance, high blood alcohol



**Fig. 5.** Approaching index of the feeding area ± SEM of the zebrafish during 8 days of the conditioning test using food as reinforcement. Each group was submitted to alcohol for 15 days followed by 11 days of withdraw (n = 12 for each group). Different letters indicate statistical differences between different blocks of days (RM Anova, p < 0.05).

concentration alters social behavior (Gerlai et al., 2009), reduces shoal formation (Buske and Gerlai, 2011), increases aggressiveness (Gerlai et al., 2000), and compromises risk perception (Gerlai et al., 2000, 2009). Alcohol is a CNS depressant drug (Charness et al., 1989) and its inhibitory action reduces reflexive and cognitive processes (Campbell et al., 2013; Koike and Sobue, 2006). In long term use, alcohol provokes severe learning and memory problems (Gomez et al., 2013; Pittman and Lott, 2014; Romero et al., 2013). The harmful effects were supported in our study, in which high doses of alcohol (0.25% and 1.00%) interfered with the cognitive ability of learning.

In contrast, low alcohol dose (0.10%) did not impair learning and allowed fish to associate the unconditioned stimulus two days before the control group (0.00% alcohol). According to Gerlai et al. (2000), fish swimming activity increases at low alcohol doses and decreases at high alcohol doses, which could be an explanation for why we see such a fast approach to the feeding area in the 0.10% group. However, our dispersion analysis showed no differences in motor pattern among alcohol exposed groups, reinforcing the idea that zebrafish improved learning purely by the effect of the low dose of alcohol, which corroborates studies of Ruitenberg et al. (2002) and Weyerer et al. (2011). Although we have observed differences in fish dispersion for the chronic alcohol exposed groups (0.25% was closer to the feeding area before light stimulus), the 0.25% group showed worse learning performance than control and 0.10% groups.

A few mechanisms have already been proposed to explain the beneficial effects of subclinical alcohol doses on learning. Kaley and During (2007) suggest that chronic consumption of low doses of alcohol improves learning in rats by increased expression of receptors N-methyl-D-aspartate (NMDA) in the hippocampus. These authors attributed it to a state of adaptive neuroprotection similar to the condition known as “preconditioning” that can be started by subtoxic doses of a variety of potentially damaging stimuli (Dirnagl et al., 2003). In fish, some stressors seem to affect cell proliferation in the hippocampus (i.e. the dorsal telencephalon – Friedrich et al., 2010) (Gould et al., 1997; Mirescu and Gould, 2006; von Krogh et al., 2010), but the effect of low alcohol doses is still unclear.

Aside from the cognitive effects of alcohol, Cachat et al. (2010) showed that withdrawal also affects the zebrafish. It is known that completely stopping the excessive use of alcohol may allow recovery of cognitive processes over time (Brown et al., 2001). However, recovery depends on a variety of different aspects such as the brain region affected, the duration of drug exposure and the extent of injury (Hohmann et al., 2000). After prolonged substance abuse the damage can also be irreversible, i.e. Wernicke–Korsakoff syndrome (Hohmann et al., 2000). In our study, the alcohol exposure period was probably not long enough to trigger large neuronal loss, therefore facilitating cognitive function recovery after a short period of abstinence. Crews and Nixon (2009) suggested that the stimulation of NMDA receptors and pCREB transcription favors neuroplasticity and neurogenesis return during the withdrawal period. However, additional studies are needed to define in which doses and when these effects could be observed.

In regard to the addictive properties of alcohol, we observed that acute and chronic exposure to 0.25 and 1.00% alcohol increased place preference in zebrafish, indicating drug-seeking behavior. This result is consistent with Brennan et al. (2011) and Mathur et al. (2011), who proposed that a single exposure is sufficient to cause addiction for up to 4 weeks. These authors used only high alcohol doses (1.00% and 1.50%) and related the addictive response to an altered dopaminergic secretion in the brain, that is a positive reinforcement response to drugs of abuse (O'Brien and Gardner, 2005). However, since exposure to 0.10% alcohol did not induce to drug seeking behavior, it seems that low alcohol doses do not affect the secretion of dopamine to a large extent.

Although we suggest some beneficial effects of low alcohol use, there is no safe amount of consumption recommended or medical use of alcohol presently known. Moreover, recent studies indicate that

even low alcohol ingestion seems to be related to cancer development in breast, liver and intestine (Zhao et al., 2012). On the other hand, alcohol overdose may have the opposite effects, promoting wide neurodegeneration (Bittencourt, 2000; Koob, 1992; Maciel and Kerr-Corrêa, 2004). Thus, even if one could keep a moderate level of alcohol use for a long period of time, only a single ingestion of a large amount could nullify the benefits of years of controlled consumption.

Finally, our study confirms the importance of zebrafish as a model for drug throughput screening. For future studies, we suggest the use of zebrafish to search for paradigms to reverse drug seeking behavior, such as punishment or reinforcement associated to withdrawal in order to weaken the brain reward systems. Also, it would be important to invest in techniques that show changes in the brain (neurotransmitters, proteins, neuroplasticity) caused by different doses of alcohol, in order to better understand the effect of low alcohol doses on learning showed here and the deleterious effects of high alcohol doses.

## 5. Conclusion

While the chronic use of low alcohol dose (0.10%) did not cause drug seeking behavior and positively interfered with the zebrafish conditioning performance, high alcohol dose exposure (0.25% and 1.00%) was shown to be highly damaging, even in acute or chronic use. On the other hand, the withdrawal treatment group seems to allow for some recovery for learning. Therefore, we suggest that low alcohol use may have altered neuronal communication, plasticity or intracellular pathways that culminate in improving learning. Overall, studies focusing on alcohol effects in the brain are needed and the use of the drug even in low dose is not recommended.

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