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Dose-Dependent Effects of Alcohol on Seeking Behavior and Memory in the Fish *Betta splendens*

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The present study tested the effects of alcohol on seeking behavior and memory in the Siamese fighting fish *Betta splendens*. We tested behavior using 5 alcohol concentrations: .00%, .10%, .25%, 1.00%, and 1.50% (vol/vol%). Drug seeking was tested using a conditioned place preference (CPP) paradigm, with a single 20-min exposure to alcohol. The effect of alcohol on memory was tested using a T-maze protocol with acute (20 min/day for 5 days) and chronic (20 min/day for 20 days) alcohol exposure and after alcohol withdrawal (20 min/day alcohol exposure for 15 days + water exposure). In the CPP test, the higher acute alcohol doses (1.00 and 1.50%) induced seeking behavior, but the lower (.10%) and medium (.25%) doses did not. When the fish were tested after 37 days of alcohol exposure, the higher-dose groups still exhibited seeking behavior, indicating that these doses may have caused drug addiction. In the memory test, we observed a dose-dependent pattern with both the acute and chronic treatments. High alcohol doses (1.00 and 1.50%) impaired memory, and low alcohol doses (.10%) caused an anticipatory response. The withdrawal group did not exhibit differences in memory, suggesting some capacity for recovery. The low alcohol doses did not impair memory or cause drug seeking, whereas the high doses affected memory and caused prolonged seeking behavior. Therefore, a dual effect of alcohol was corroborated by our data, and *Betta splendens* may be an adequate animal model for high-throughput screening with alcohol.

Keywords: conditioned place preference, abstinence, seeking behavior, tolerance, *Betta splendens*

Among substances of abuse, alcohol is the most widely consumed and responsible for numerous behavioral alterations, including psychological addiction (Givens & McMahon, 1995) and memory loss (Uecker & Nadel, 1996). Addiction is a complex psychiatric disorder that involves compulsive drug-seeking behavior even after prolonged abstinence (Brennan et al., 2011). Drug-induced conditioned place preference (CPP) is a noninvasive and simple procedure that can be used to study the reinforcing properties of drugs of abuse (Darland & Dowling, 2001; Ninkovic & Bally-Cuif, 2006; Bretaud et al., 2007; Kily et al., 2008). The loss of control of behavior, referred to as compulsive drug seeking, is associated with increased dopaminergic transmission in the mesolimbic system (Rink & Wullimann, 2002). However, the genetic and neuroethological bases of seeking behavior require a thorough understanding to develop pharmacological and psychological therapies for the treatment of drug seeking/addiction.

In addition to alcohol's addiction potential, impairments in memory are a common symptom when the drug is taken in high amounts, referring to the loss of the ability to record information. Indeed, heavy drinking can cause extensive effects on the brain, from simple slips in memory to a condition of permanent dementia called Wernicke Korsakoff syndrome (Sav-

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age, Candon, & Hohmann, 2000). Several studies (Fuller & Hiller-Sturmhöfel, 1999; Vengeliene, Bilbao, Molander, & Spanagel, 2008; Gerlai, Chatterjee, Pereira, Sawashima, & Krishnannair, 2009) indicate the need to study various alcohol concentrations to determine dose-effect functions and the way the drug acts within the brain (Gerlai et al., 2009). However, studies that evaluate the effects of low-dose alcohol on seeking behavior and cognitive responses are lacking.

Although research that uses rodents and primates that are exposed to alcohol facilitates human translation, the complexity of mammalian neural mechanisms makes such studies difficult. The teleost fish exhibits learning and memory performance that is comparable to mammals (Odling-Smee & Braithwaite, 2003; Yoshida & Kondo, 2012). These fish also share organizational and functional characteristics of the central nervous system with other vertebrates (Kaslin & Panula, 2001; Holzschuh, Ryu, Aberger, & Driever, 2001; Teraoka et al., 2004; Kaslin, Nystedt, Ostergård, Peitsaro, & Panula, 2004; McLean & Fetcho, 2004; Mueller, Vernier, & Wullimann, 2004; Faraco et al., 2006; Prober, Rihel, Onah, Sung, & Schier, 2006; Yokogawa et al., 2007; Yoshida & Hirano, 2010) but have simpler neural pathways, making them adequate models for the study of brain mechanisms (Gerlai, Lahav, Guo, & Rosenthal, 2000; Gerlai, 2002; Pather & Gerlai, 2009; Souza & Tropepe, 2011; Karnik & Gerlai, 2012). Furthermore, fish can learn and remember environmental signals and events (Odling-Smee & Braithwaite, 2003), processes that can be affected by the actions of drugs of abuse, such as alcohol.

The fish *Betta splendens*, which is native to small streams and lakes in Southeast Asia, utilize advanced spatial/motor memory to recognize locations where food is available, locate conspecifics (opponents and mates), and avoid predators (Braddock & Braddock, 1955; Roitblat, Tham, & Golub, 1982; Verbeek, Iwamoto, & Murakami, 2008). Such ecological and social features appear to have favored the memory ability of this species. Therefore, we used *B. splendens* as a model to better understand the effects of alcohol on performance in two memory paradigms: conditioned place preference (CPP) and spatial memory in a T-maze. Our first aim was to evaluate seeking behavior caused by alcohol using a CCP model, in which alcohol was used as the reward. Our second aim was to study the effects of alcohol on memory, in which alcohol was not associated with the task but affected how the animals performed it.

Method

Animals

The present study used adult *Betta splendens* obtained from a local fish farm and kept in storage tanks (50 × 40 × 30 cm, 50 L, 1 fish/L) in the Fish Laboratory (Departamento de Fisiologia, UFRN, Natal, Brazil). The aquarium water was maintained at 28 °C, with constant oxygenation and 30% water exchange every 10 days. The photoperiod was set at a 12-hr light–dark cycle. The animals were fed daily with a commercial diet (38% protein and 4% fat, Nutricom Pet) ad libitum. Only female fish were used because they are less aggressive and more explorative in the absence of males (Giannecchini, 2010). All of the animal procedures were performed with permission from the Ethical Committee for Animal Use of the Universidade Federal do Rio Grande do Norte (CEUA 025/2012).

Apparatus and Procedures for Seeking-Behavior Test

The experimental design involved the evaluation of seeking behavior induced by different doses of alcohol with acute treatment in a conditioned place preference paradigm (CPP) adapted from the procedures used by Brennan et al. (2011) and Mathur, Berberoglu, and Guo (2011).

The testing apparatus was a 15 L aquarium that was divided in half with an opaque glass divider (shuttle box, 40 × 25 × 20 cm). The bottom of each side of the tank had different visual cues. One side was completely white, and the other had a black and white grid (2 × 2 cm). The lateral walls of the tank were covered in white. Initial preference was determined for each fish by individually introducing them in the shuttle box. After a 2-min acclimation period, behavior was recorded from above using a Sony Digital Video Camera Recorder (DCR-SX45) for 5 min. The videos were analyzed using the ANY-maze™ Video Tracking Sys-

tem, which recorded the time spent on each side of the shuttle box. The side where the fish spent more than 60% of the total time was considered the preferred side. The fish were maintained in individual aquaria (700 ml) that allowed visual contact between them (to avoid isolation stress) until the conditioning test.

The day after the initial determination of place preference, the fish were exposed to alcohol in a single 20-min period (acute exposure) on the least preferred side of the aquarium. We used a smaller aquarium (20 × 20 × 10 cm, 2 L) that was placed inside the shuttle box that allowed the fish to see the bottom. The fish were then restricted to the preferred side for 20 min in freshwater. Regardless of the initial preference, the fish were always first exposed to alcohol in the least preferred place and then placed in freshwater on the preferred side. This procedure ensured that the fish were exposed to the drug only in the least preferred location. Following this procedure, each fish was removed to freshwater in individual aquaria (700 ml).

Alcohol concentrations of .10%, .25%, 1.00%, and 1.50% (vol/vol%) were achieved by diluting 99.9% alcohol P.A. in water. The control group was always kept in .00% alcohol ($n = 9$). Fish that were subjected to acute alcohol treatment received alcohol only the day after the initial determination of place preference (.10%, $n = 9$; .25%, $n = 8$; 1.00%, $n = 10$; 1.50%, $n = 10$).

To define the reinforcing effects of alcohol, the place preference of each fish was tested on the following 5 days and on Day 37 after the single exposure. The animals were recorded for 5 min to observe possible changes in preference. All fish tracking was performed using the ANY-maze™ Video Tracking System.

Apparatus and Procedures for Memory Test

The experimental design involved evaluation of the effects of different alcohol doses on performance in a spatial memory task. We used a simple T-maze without cues. The apparatus was made of polyvinyl chloride, with an exit only in one arm. The fish had to remember whether the left or right arm led to the exit. The apparatus was placed inside a larger tank (50 × 50 × 30 cm) that had all of its walls covered with black fabric to avoid external interference.

Three experimental groups were tested: (a) acute treatment (alcohol exposure for 20 min/day during the 5 days of the test; $n = 12$; the fish were under the effects of alcohol throughout the test); (b) chronic treatment (alcohol exposure for 20 min/day for 15 days before + 5 days of the test; $n = 12$; the animals were exposed to alcohol at least 2 h before beginning the task to ensure that they were not under the effects of alcohol during the test, according to alcohol measurements by Tran & Gerlai, 2013); (c) withdrawal treatment (the fish were pretreated with alcohol for 20 min/day for 15 consecutive days, and after 2 days without alcohol exposure, the task began; $n = 12$).

The following alcohol doses were used: .00% (control), .10%, .25%, 1.00%, and 1.50%. Alcohol exposure was performed every evening by transferring the fish to an aquarium (20 × 20 × 10 cm, 2 L) that contained the dose previously prepared for the specific treatment. Alcohol exposure always lasted 20 min. Afterward, the fish were returned to their residence aquarium with clean freshwater until the next alcohol exposure. The control group was moved to another aquarium with only water during the same period of alcohol exposure.

For the memory test, the fish were individually placed in the maze, and the time to exit was recorded. The animals were not fed for 2 days before the test phase. During the 5 days of testing in the T-maze, the fish received only one adult *Artemia salina* as a reward for exiting the maze. No other food was supplied during this period.

While performing the memory tests, we also determined whether the alcohol doses interfered with locomotion, which could affect performance in the maze. Ten female *B. splendens* were exposed to alcohol for 20 min at each concentration (.00%, .10%, .25%, 1.00%, and 1.50%) and individually placed in tanks (40 × 25 × 20 cm, 15 L), and swimming behavior was recorded. The average and maximum swim speeds were analyzed for 10 min using the ANY-maze™ Video Tracking System.

Statistical Analysis

For the seeking-behavior test (CPP), the percentage of time spent on each side of the shuttle box was compared between initial preference and the days after alcohol exposure using

repeated-measures analysis of variance (ANOVA) because the data met the criteria in the normality and equal variance tests. Significant effects and interactions in the ANOVA were followed by the Student-Newman-Keuls post hoc test. A probability level of $p < .05$ was used as an index of statistical significance.

For the memory test, performance on the test days was compared using repeated-measures ANOVA for chronic treatment and the Friedman repeated-measures ANOVA for acute and withdrawal treatments, depending on the normality and homoscedasticity of the data. The performance of the fish with different treatments was compared using one-way ANOVA for chronic treatment and the Kruskal-Wallis test for acute and withdrawal treatments to determine differences in the effects caused by the different alcohol doses. The total time to exit the maze (sum of the 5 days) was also analyzed using ANOVA and the Kruskal-Wallis test. The average and maximum swim speed data were compared using the Kruskal-Wallis test. In all cases, $p < .05$ was used as the reference value.

Results

Seeking-Behavior Test

Acute alcohol exposure dose-dependently changed the fish's initial preference (see Figure 1). We found that 25 fish initially chose the white side of the shuttle box, and 21 fish preferred the grid side. The control group (.00%) did not exhibit a change in preference throughout the test (repeated-measures ANOVA, $F = 1.96$, $p = .09$; Figure 1a). The percentage of time spent on the nonpreferred side of the shuttle box was 35.7% ± 9.7% in the initial preference test. During the 5 days after manipulation (instead of alcohol exposure), the percentage of time spent on the nonpreferred side did not increase by more than 46%.

Fish that were exposed to .10% alcohol changed their initial preference only on Day 3 after alcohol exposure (repeated-measures ANOVA, $F = 2.63$, $p = .032$; Figure 1b), but we did not observe differences on the other days. With .25% alcohol, the fish's preference differed between Days 2 and 37 but not on the other days (repeated-measures ANOVA, $F = 2.67$, $p = .027$; Figure 1c). Fish that received alcohol at a concentration of 1.00% changed their initial preference on all days after alcohol exposure, with the exception of Day 2 (repeated-measures ANOVA, $F = 3.51$, $p = .01$; Figure 1d). The group that was exposed to 1.50% alcohol completely changed their initial preference and remained in the noninitially preferred compartment on the 5 days postalcohol and Day 37 (repeated-measures ANOVA, $F = 8.93$, $p < .001$; Figure 1e).

Memory Test

Acute treatment. All of the groups exhibited a decrease in the time to exit the T-maze throughout the test days (Friedman test; .00%: $\chi^2 = 23.1$, $p = .001$; .10%: $\chi^2 = 35.4$, $p = .001$; .25%: $\chi^2 = 15.0$, $p = .005$; 1.00%: $\chi^2 = 30.1$, $p < .001$; 1.50%: $\chi^2 = 29.7$, $p < .001$; Figure 2a). The comparison between the acute doses on the same test days indicated significant difference between groups on Day 1 (Kruskal-Wallis, $H = 19.6$, $p < .001$), Day 3 (Kruskal-Wallis, $H = 17.8$, $p < .001$), and Day 5 (Kruskal-Wallis, $H = 17.3$, $p = .002$). On Day 3, fish that received 1.50% alcohol treatment exhibited the longest time to exit the maze, whereas on Day 5, fish that were exposed to .10% alcohol exited the maze faster than the other groups (Figure 2a).

Chronic treatment. All of the groups exhibited a decrease in the time to exit the maze throughout the test days (repeated-measures ANOVA; .00%: $F = 63.6$, $p = .001$; .10%: $F = 16.2$, $p = .001$; .25%: $F = 14.92$, $p < .001$; 1.00%: $F = 5.5$, $p = .013$; 1.50%: $F = 26.6$, $p < .001$; Figure 2b). The comparison between doses revealed differences in task performance on Day 1 (ANOVA, $F = 3.3$, $p = .02$) and Day 5 (ANOVA, $F = 4.1$, $p = .006$) but not Day 3 (ANOVA, $F = 2.17$, $p = .084$). On both Days 1 and 5, the .10% alcohol group was the fastest to exit the maze, and the 1.50% alcohol group was the slowest.

Withdrawal treatment. All of the groups exhibited a decrease in the time to exit the maze during the test days (Friedman test; .00%: $\chi^2 = 16.8$, $p = .002$; .10%: $\chi^2 = 22.9$, $p < .001$; .25%: $\chi^2 = 31.7$, $p < .001$; 1.00%: $\chi^2 = 10.7$, $p = .03$; 1.50%: $\chi^2 = 29.7$, $p < .001$; Figure 2c). The comparisons between doses on the same test day showed that the fish that were withdrawn from 1.00% alcohol were the slowest to accomplish the task on Day 5 (Kruskal-

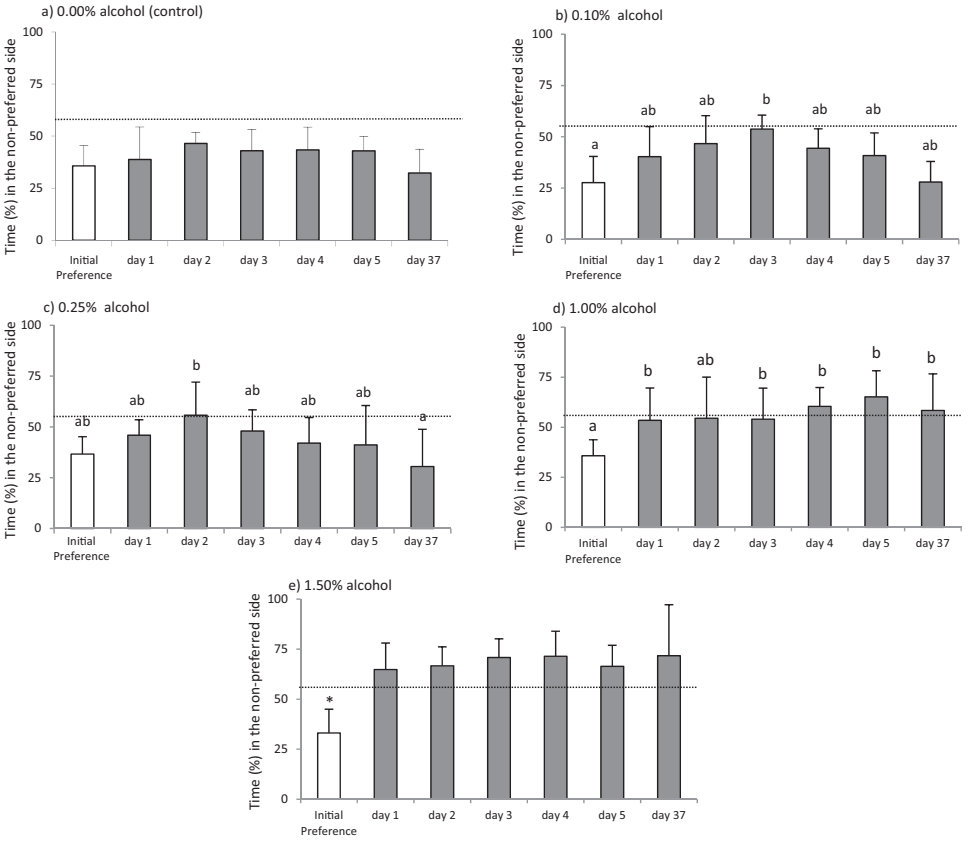

Figure 1. Conditioned place preference induced by acute alcohol exposure. The bars indicate the mean ± *SD* percentage of time spent on the less preferred side in a shuttle box (black and white grid vs. total white). Initial preference was tested before acute alcohol exposure (white bars), and Days 1–5 and 37 indicate the time spent on the less preferred side after alcohol exposure. The fish were exposed only once to different alcohol doses (control, $n = 9$; .10%, $n = 9$; .25%, $n = 8$; 1.00%, $n = 10$; 1.50%, $n = 10$) for a 20-min period on the less preferred side. The dashed line represents the percentage used (60%) to indicate preference. Same letters above the bars indicate no significance, and different letters or the asterisk (*) indicates significance (repeated-measures ANOVA and Friedman test, $p < .05$).

Wallis, $H = 18.0$, $p = .001$; Figure 2c). The total time spent inside the maze during all 5 days of testing is shown in Figure 2d. A significant difference was found between doses with acute and chronic treatment (acute, Kruskal-Wallis, $H = 33.2$, $p < .001$; chronic, repeated-measures ANOVA, $F = 6.68$, $p < .001$), in which the groups that were exposed to the higher doses exhibited the longest time to exit the maze. The withdrawal groups exhibited no differences among doses (Kruskal-Wallis, $H = 3.3$, $p = .49$). The comparison between treatments (acute, chronic, and withdrawal) using the same alcohol dose revealed differences between chronic and withdrawal treatment at alcohol concentrations of .10% (ANOVA, $F = 3.38$, $p = .03$) and .25% (ANOVA, $F = 4.15$, $p = .03$), whereas the acute alcohol doses of 1.00% and 1.50% differed from the chronic and withdrawal treatments (1.00%, Kruskal-Wallis, $H = 17.6$, $p = .001$; 1.50%, ANOVA, $F = 7.78$, $p = .002$).

Locomotor Activity

The alcohol treatments did not alter swimming activity. The average speed was .01 ± .002 m/s in the .00% group, .01 ± .005 m/s in the .10% group, .02 ± .014 m/s in the .25%

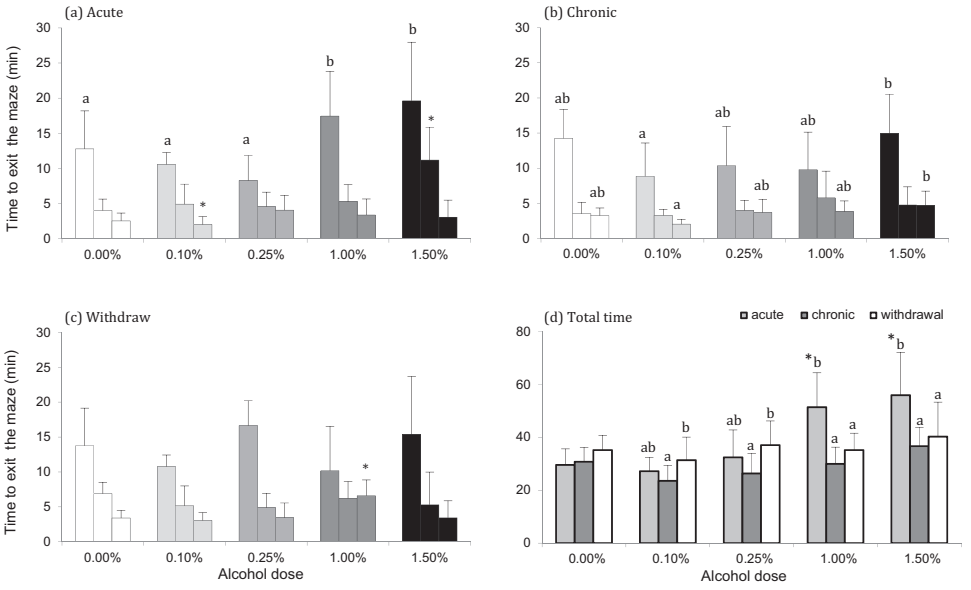

Figure 2. The time to exit the maze in a motor memory task in *Betta splendens*. The fish were exposed to alcohol concentrations of .0% (control), .10%, .25%, 1.00%, and 1.50% with three different treatment regimens: (a) acute treatment (alcohol exposure before testing in the maze; $n = 12$), (b) chronic treatment (alcohol exposure for 15 days before and during the days of testing; $n = 12$), and (c) withdraw treatment (alcohol exposure for 15 days before the tests but not during the tests; $n = 12$). The bars show the mean time to exit the maze on Days 1, 3, and 5 out of 5 days of training. (d) Total time that the fish remained inside the maze for 5 consecutive days. Different lowercase letters or asterisks (*) indicate significant differences between alcohol doses with the same treatment (one-way ANOVA, $p < .05$).

group, .01 ± .004 m/s in the 1.00% group, and .01 ± .004 m/s in the 1.50% group. No significant difference in average swim speed was found between groups (Kruskal-Wallis, $H = 7.2$, $p = .12$). The maximum speed was .29 ± .08 m/s in the .00% group, .24 ± .17 m/s in the .10% group, .35 ± .4 m/s in the .25% group; .21 ± .07 m/s in the 1.00% group, and .27 ± .3 m/s in the 1.50% group. No significant difference was found in maximum speed between groups (Kruskal-Wallis, $H = 6.3$, $p = .17$).

Discussion

We found that alcohol dose-dependently promoted seeking behavior in *Betta splendens*. The lowest dose of the drug (.10%) did not change place preference, whereas the highest dose (1.50%) completely altered basal preference up to 37 days after exposure. We also found that *Betta splendens* exhibited spatial memory in a T-maze without environmental cues, but its performance was significantly affected by alcohol. Acute higher doses (1.00% and 1.50%) impaired memory. Chronic alcohol exposure resulted in performance that was similar to withdrawal (see Figure 2).

Many studies have shown that fish have advanced learning and memory capacity. For example, the goldfish *Carassius auratus* is able to remember the spatial position of three different feeding sites that are distributed in an arena (Pitcher & Magurran, 1983). The zebrafish *Danio rerio* uses visual cues for guidance in learning tasks (Karnik & Gerlai, 2012). The Siamese fighting fish *Betta splendens* shows good performance in an eight-arm radial maze (Roitblat et al., 1982). Thus, our study corroborated the fact that fish utilize cognition to exit a maze and receive a reward. The memory task that was used in the present study requires the animal to remember where the exit of the maze is located (e.g., left or right side).

However, when *B. splendens* was exposed to high alcohol doses, the fish presented apparent difficulty exiting the maze. One brain region that is particularly sensitive to alcohol is the cerebellum (Bauer-Moffett & Altman, 1975; Clarren, Alvord, Sumi, Streissguth, & Smith, 1978; Pierce, Goodlett, & West, 1989; Goodlett, Marcussen, & West, 1990; Sowell et al., 1996). The cerebellum is responsible for many functions, including some cognitive and emotional functions (Yoshida, Okamura, & Uematsu, 2004; Rodriguez et al., 2005; Wolf, Rapoport, & Schweizer, 2009; Yoshida & Kondo, 2012), but it also plays an important role in motor control (Ito, 1984; Yoshida et al., 2004), which is one of the first functions damaged by alcohol intake (Thomas, Goodlett, & West, 1998). Some studies suggest that alcohol directly affects cerebellar Purkinje cells (Phillips & Cragg, 1982; Pierce, Serbus, & Light, 1993; Napper & West, 1995), causing a decrease in number, which is likely related to motor deficits caused by alcohol exposure. Gruol and Curry (1995) proposed that alcohol damages cerebellar Purkinje cells through its effect on intracellular calcium concentrations elicited by glutamatergic neurotransmission. This potential mechanism is not completely clear, and other studies that evaluate the effects of alcohol on motor function may help better understand how the drug acts in the brain.

All vertebrates have a cerebellum that varies in size and shape (Meek, 1992), but it has very similar structural patterns and cytoarchitectural organization (Nieuwenhuys, ten Donkelaar, & Nicholson, 1998; Butler & Hodos, 2005). Although most knowledge about cerebellar function has been derived from mammalian studies, fish show comparable potential and simpler neural pathways that make them an adequate model for brain research (Gerlai et al., 2000; Gerlai, 2002). Lesions of the cerebellum in rats have been reported to prevent the acquisition of spatial information, in which animals exhibit defective exploration patterns and peripheral circling in spatial tasks, such as the water maze and T-maze (Lalonde & Botez, 1986; Goodlett, Nonneman, Valentino, & West, 1988; Petrosini, Leggio, & Molinari, 1998; Rondi-Reig, Le Marec, Caston, & Mariani, 2002). In fish, some studies have shown that damage to the cerebellum impairs conditioned cardiac responses in classical fear conditioning paradigms (Yoshida et al., 2004; Yoshida & Hirano, 2010) and stereotyped and inefficient exploratory behavior and inaccuracy in achieving task goals that require spatial memory (Gómez, Durán, Salas, & Rodríguez, 2010). Although *Betta splendens* had an intact cerebellum in the present study, our results clearly suggest that the ability to reach the maze exit was decreased by alcohol. Thus, we believe that the behavioral disadvantage caused by the drug may have occurred because of its effects in the brain, mainly the cerebellum.

The cerebellum is an important area because it is required for motor function and contributes to different sensorial, cognitive, and emotional functions (Sullivan, Deshmukh, Desmond, Lim, & Pfefferbaum, 2000; Álvarez et al., 2002; Rodríguez et al., 2005; Yoshida & Hirano, 2010). The cerebellum is also highly sensitive to alcohol exposure (Sowell et al., 1996). Alcohol is one of the most effective drugs in destroying brain tissue (Willoughby, Sheard, Nash, & Rovet, 2008; Norman, Crocker, Mattson, & Riley, 2009) and impairing different types of memory (Matthews, Simson, & Best, 1995; Uecker & Nadel, 1996; Hamilton, Kodituwakku, Sutherland, & Savage, 2003). In fact, our data corroborate these findings, in which high doses of alcohol affected the fishes' performance in finding the T-maze exit. However, the precise effects of alcohol on the cerebellum and other brain areas still need to be confirmed in future studies.

Although we showed that animals that were exposed to acute 1.50% alcohol exhibited a decrease in the time to complete the task on the final day (Figure 2a), this response may have been more related to the harmful effects of alcohol with the first exposure (which caused navigation difficulties) than related to memory. One could argue that alcohol affects motion, and high alcohol doses make the animals too slow to complete the task. This possibility, however, may be discarded in the present study because the average and maximum swim speeds were not significantly different between groups. The alcohol doses that were used in the present study appear to have acted mainly in areas related to egocentric spatial memory more than in areas related to motion. Acute alcohol exposure potentiates γ-aminobutyric acid-A ($GABA_A$) receptor function (Mehta & Ticku, 1988) and inhibits

N-methyl-D-aspartate receptor function (Lovinger, White, & Weight, 1989), which has been suggested to inhibit memory formation (Morrisett & Swartzwelder, 1993; Schummers & Browning, 2001).

The main difference in performance in fish that were chronically exposed to alcohol was observed between the .10% and 1.50% doses. Fish that were exposed to the other doses (.25% and 1.00%) exhibited memory performance that was comparable to controls (.00%). Chronic alcohol exposure appeared to promote tolerance to the drug. This result is consistent with Boulouard, Lelong, Daoust, and Naassila (2002), who showed that chronic alcohol administration produces tolerance to the adverse effects of acute alcohol exposure in rats. According to these authors, tolerance can be partly attributed to cellular and molecular adaptations of glutamate and GABA neurotransmissions. However, an intriguing finding was the harmless effect of chronic exposure to .10% alcohol. We suggest that the .10% concentration may have induced a low level of stress in the central nervous system and thus may have increased cholinergic neurotransmission to allow the fish to complete the task. This possibility is consistent with the suggestions of Ruitenberg et al. (2002) but yet to be confirmed.

Our results also showed that 7 days of withdrawal had no effect on memory. Fish that were subjected to withdrawal performed similarly to control fish (.00%). One hypothesis could be that the *B. splendens* exhibits tolerance to alcohol and recovers more efficiently than other animals, possibly because of the high neurogenesis that is observed in fish (Grandel, Kaslin, Ganz, Wenzel, & Brand, 2006). Other reports indicate that the harm caused by alcohol abuse can be reversed by suspending its use (Naranjo, Knoke, & Bremner, 2000). Neuroimaging studies have confirmed that brain dysfunctions that are related to alcohol may be reversible over the time of withdrawal (Tapert et al., 2001). However, differences in recovery may be observed according to the affected brain region, duration of drug exposure, and extent of damage (Savage et al., 2000). The damage may be irreversible after years of substance abuse, such as in Wernicke Korsakoff alcoholic dementia (Savage et al., 2000). In the present study, the alcohol exposure period was probably not sufficiently long to trigger large neuronal loss, thus facilitating the recovery of cognitive function after a short period of abstinence. However, brain abnormalities after prolonged alcohol use still require further investigation that focuses on the duration of drug use and the withdrawal period.

Furthermore, we found that the low dose of alcohol did not generate any seeking behavior, whereas higher doses led to prominent changes in behavior. In mammals, the mesolimbic dopamine system plays an important role in the positive reinforcing effects of drugs of abuse (O'Brien & Gardner, 2005). In the present study, we found that a single exposure to 1.00% and 1.50% alcohol changed place preference up to 37 days after exposure, characterizing drug-seeking behavior. Our observation supports the results reported by Brennan et al. (2011) and Mathur et al. (2011). These authors proposed that only one exposure to the drug is sufficient to cause addiction, possibly because of altered dopamine secretion patterns, which does not appear to occur for relatively low doses (O'Brien & Gardner, 2005). Exposure to .10% alcohol did not induce seeking behavior, and low alcohol doses do not appear to affect brain dopamine secretion to a large extent. Thus, we believe that a threshold of alcohol use triggers seeking behavior and the future development of addiction. Thompson, Stockwell, and MacDonald (2012) measured the risk of developing addiction in adolescents and also found an increasing linear relationship between addiction and the amount of alcohol consumed in a single episode.

Finally, the present results indicate that the Siamese fighting fish can be a useful model for high-throughput screening with alcohol. Alcohol can either positively or negatively interfere with behavior, depending on the dose tested. Low doses of alcohol may not affect memory or cause changes in seeking behavior, whereas high doses can impair cognition and cause drug seeking for prolonged periods of time. Future studies should investigate the effects of alcohol on brain tissue (neurotransmitters, proteins, and neuroplasticity) to better understand whether low doses are actually harmless as demonstrated herein and why high doses are so deleterious.

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