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Does Acute Alcohol Exposure Modulate Aggressive Behaviors in the Zebrafish (*Danio rerio*), or is the Bark Worse than the Bite?

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Previous research reports that acute alcohol exposure disrupts shoaling behavior in the zebrafish. The purpose of these studies is to better understand how acute alcohol exposure (0%, 0.125%, 0.25%, 0.5%, and 1.0%) alters zebrafish behavior. The effects of alcohol on aggressive behaviors in humans have been widely researched. Previous research from this lab has shown a bimodal effect of alcohol on shoaling behavior in zebrafish, with 0.5% and 2.0% (v/v) disrupting shoaling while 1.0% and 1.5% showing no direct effect. Because shoaling is a social behavior and is altered during acute alcohol exposure, aggressive behavior between fish should be addressed. In this series of experiments we explored alcohol's effects on aggressive behaviors. In order to address a possible role for alcohol induced aggression as it relates to shoaling we chose to examine the effects of acute alcohol exposure on zebrafish pairs. Fish were assessed during initial encounters occurring in our testing apparatus during acute alcohol exposure. Results show a change in biting as a function of all doses. Acute alcohol exposure (0.5%) also decreases overall occurrences of chasing and retreating but may increase the duration of each bout. Lastly in a separate experiment we looked at blood alcohol levels as a result of acute alcohol exposure.

Acute alcohol exposure has been shown to modulate a variety of behaviors in adult zebrafish. Specifically, locomotor activity and shoal cohesion are two behaviors that can change when zebrafish are acutely exposed to alcohol (Gerlai, Lahav, Guo, & Rosenthal, 2000). Data collected with rodent, human and primate subjects suggest that induced alterations of motor function follow a characteristic inverted U-shaped function, with intermediate doses of ethanol enhancing locomotor activity and higher doses suppressing it (for reviews see Barr, Schwandt, Newman, & Higley, 2004; Fogarty & Vogel-Sprott, 2002; McBride & Li, 1998).

The effects of alcohol on aggressive behaviors in humans have been widely researched. According to a meta-analysis by Bushman and Cooper entitled "Alcohol and Human Aggression" (1990), alcohol induces aggressive behavior in humans as much as any other social or non-social behavior.

Acute alcohol exposure influences social shoaling behavior in zebrafish and has been shown to influence aggression in other species, including humans. Thus, it is possible that aggression may play a role in the disruption of social behavior seen in zebrafish under acute alcohol exposure, yet this relationship has not been investigated. In order to address this, we chose to examine the effects of acute alcohol exposure on aggression in zebrafish pairs.

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Prior to testing, fish were individually housed in home tanks. Fish were assessed throughout initial first-time encounters. This occurred in our testing apparatus during acute alcohol exposure. Dependent measures of aggression (occurrences and duration of chases, occurrences and duration of retreats, and biting) were based on a behavioral model of dominance and subordinance by Larson, O'Malley, and Melloni Jr. (2006). Our question was does acute alcohol exposure (0%, 0.125%, 0.25%, 0.5%, and 1.0%) alter shoaling behavior by mediating aggression in the zebrafish?

Remarkably, our results support a decrease in biting behavior as a function of all doses. More specifically, acute exposure to a 0.5% alcohol concentration decreased overall occurrences of chasing and retreating but paradoxically there is a trend for the increased duration of each bout. This particular dose was of special interest, because it has been widely reported to disrupt shoaling (Dlugos & Rabin, 2003; Gerlai, 2003; Gerlai et al., 2000).

As one surveys the zebrafish literature, there is very little information on blood alcohol concentrations (BAC) that are the result of acute exposure. We infer from behavior changes, and predict due to water solubility and zebrafish anatomy that alcohol absorption has occurred. To this end, in a separate series of experiments we report the BAC as a function of time for acute exposure.

Method

Subjects

Adult zebrafish (*Danio rerio*), purchased from Crystal Reef in Hattiesburg, Mississippi, were housed in a community tank system (individually in 1.5 liter tanks for aggression testing and in groups of ten in 10.0 liter tanks for BAC testing; Aquatic Habitats; Apopka, Florida) at a temperature of approximately 27-C. The community system was connected to a main reservoir and contained aeration and filtration units circulating de-chlorinated H_2O. Drug exposure and testing took place during the light cycle between 8:00 a.m. and 5:00 p.m. Fish were kept on a 14 h on and 10 h off light cycle and were fed flake food twice daily (TetraMin, Blacksburg, VA). Both male and female fish were used in the experiments. One week prior to behavior testing subjects were individually housed. Experimental pairs were never housed together prior to the seven-day separation. Sixteen pairs were tested in each of the five conditions (0%, 0.125%, 0.25%, 0.5%, and 1.0%). Testing sessions lasted ten minutes and were video recorded using a Flip Mino HD video camcorder mounted to a tripod (Best Buy, online) and subsequently coded. During scoring, videos were viewed at half speed by observers blind to treatment conditions.

Aggression / pair interaction

We assessed alcohol induced behavior changes with an open field task (18cm X 18cm sectioned off from a 5 gallon aquarium, Crystal Reef, Hattiesburg Mississippi). Water in this tank was filtered, aerated and kept at a constant temperature approx. 27°C and the water level was about 6cm high. Prior to testing fish were housed separately in home cages. Based on a behavioral model of dominance and subordinance by Larson et al., (2006) aggressive behaviors measured in zebrafish pairs were: biting, chasing frequency, duration of chasing bouts, retreating frequency, and duration of retreating bouts. Chasing was operationalized as one fish moving quickly towards another over a distance of two or more body lengths. Retreating was defined as one fish moving rapidly away from another over a distance of two or more body lengths. Biting was scored when one fish bit another. Immobility was also measured as a means for identifying unwanted side effects of intoxication. Immobility displays can also be due to illness directly or indirectly related to toxicity. With the

exception of fin movement, immobility time was defined as time spent without movement in any direction. Data from these studies were tested with a one-way ANOVA and Tukey's posthoc tests.

Blood alcohol concentrations

In a separate series of experiments we measured blood alcohol concentrations in the zebrafish after acute exposure to the following doses 0.0%, 0.125%, 0.25%, 0.5%, and 1.0% using a spectrophotometer. In order to quantify how much ethanol was absorbed into the zebrafish, the project used the EnzyChrom Ethanol Assay Kit (ECET-100) from BioAssay Systems. Twelve fish per treatment group were exposed to a condition for ten minutes. Immediately afterwards fish were anesthetized with MS-222 (Tricane) and blood was extracted for processing. Twelve fish yielded approximately 50-70 microliters of blood.

Results

Aggression / pair interaction

The effects of acute alcohol exposure (0%, 0.125%, 0.25%, 0.5%, and 1.0%) were evaluated during a ten-minute trial. The most compelling effect of acute exposure was a significant dose-independent reduction of our primary dependent measure, biting [$F(4, 75) = 5.49$, $p < 0.01$]. Post hoc testing (Tukey HSD) revealed that all doses significantly differed from control. Interestingly, the dose range tested here when compared to control yielded no changes on immobility behavior [$F(4, 75) = 1.41$, $p = 0.23$] (data not shown). Figure 1 illustrates that when compared to control, the reduction of biting behavior was seen with all alcohol doses.

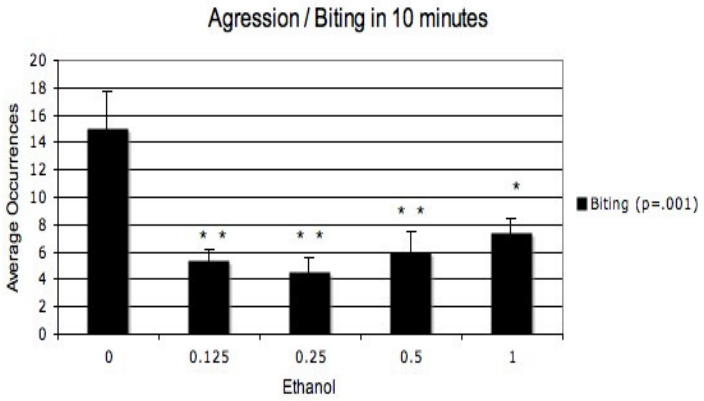

Figure 1. Effects of acute alcohol exposure on biting behavior in adult zebrafish [$F(4, 75) = 5.49$, $p < 0.01$]. Mean differences were analyzed using a one-way ANOVA and Tukey's HSD post-hoc tests were used to indicate the direction of effects where significant. Mean (±SEM) for bites for each condition is shown *$p < 0.05$ and **$p < 0.01$ ($n = 16$ pairs per treatment condition).

Acute exposure to the dose range reported here did not effect the overall time a given pair spent engaged in chasing [$F(4, 75) = 0.973$, $p = 0.44$] or retreating [$F(4, 75) = 0.798$, $p = 0.53$] behaviors. Figure 2 illustrates that when compared to control, acute alcohol exposure yielded no significant change in the

overall time spent engaged in chasing and retreating behaviors during the ten-minute test session.

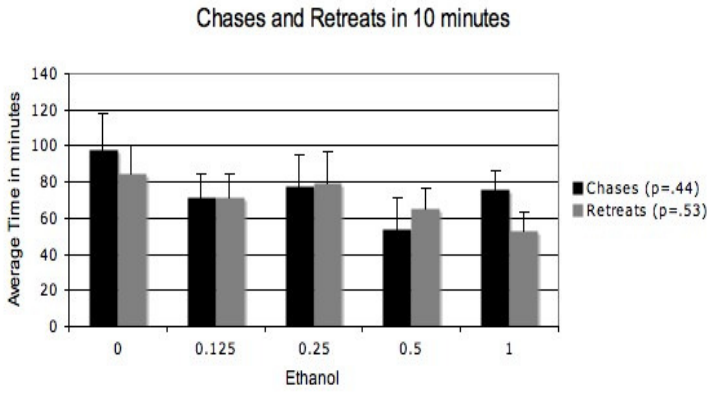

Figure 2. Non-Effects of acute alcohol exposure on overall chasing [$F(4, 75) = 0.973$, $p = 0.44$] and retreating [$F(4, 75) = 0.798$, $p = 0.53$]. Mean differences were analyzed using a one-way ANOVA. Mean (±SEM) time (minutes) for each condition is shown ($n = 16$ pairs per treatment condition).

While the *overall* time spent engaged in chasing or retreating did not change the *frequency* of chasing [$F(4, 75) = 2.52$, $p < 0.05$] and retreating [$F(4, 75) = 2.56$, $p < 0.05$] behavior (how often these behaviors occurred) was effected by acute alcohol exposure. Post hoc testing (Tukey HSD) revealed that exposure to the 0.5% dose significantly reduced the display of chasing and retreating behaviors ($p = 0.03$, and $p = 0.02$, respectively) during the ten-minute test session. Figure 3 illustrates that when compared to control, the 0.5% dose significantly reduced the occurrence of chasing and retreating between zebrafish pairs.

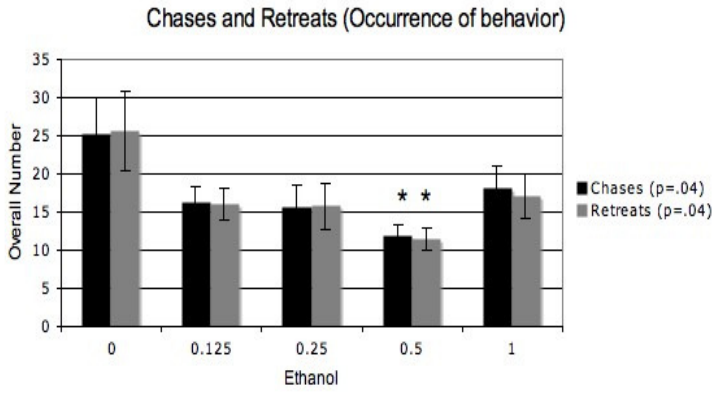

Figure 3. Effects of acute alcohol exposure on the occurrence of chasing [$F(4, 75) = 2.52$, $p < 0.05$] or retreating behavior [$F(4, 75) = 2.56$, $p < 0.05$]. Mean differences were analyzed using a one-way ANOVA and Tukey's HSD post-hoc tests were used to indicate the direction of effects where significant. Mean (±SEM) for the instances of chasing and retreating in each condition is shown $*p < 0.05$ ($n = 16$ pairs per treatment condition).

When assessing the average length of time of an episode for a fish pair engaged in a chasing and retreating bout we saw a trend towards an increase in episode length. This non-significant trend just narrowly missed significance for chasing [$F(4, 75) = 2.34$, $p = 0.06$] and retreating [$F(4, 75) = 2.42$, $p = 0.05$]. Figure 4 shows this trend.

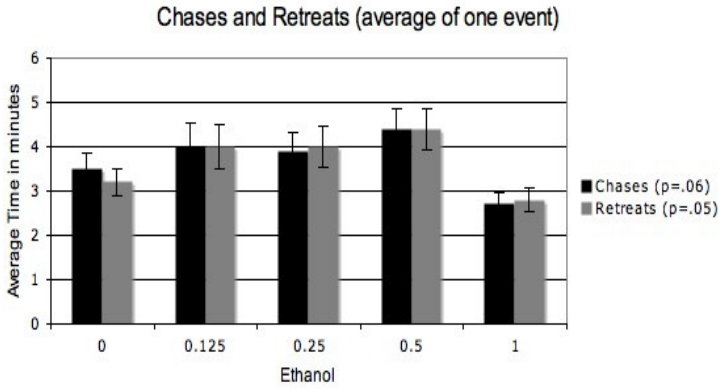

Figure 4. Nearly significant trend of acute alcohol exposure on the average time of a bout of chasing [$F(4, 75) = 2.34$, $p = 0.06$] or retreating [$F(4, 75) = 2.42$, $p = 0.05$]. Mean differences were analyzed using a one-way ANOVA. Mean (±SEM) time (minutes) for each condition is shown ($n = 16$ pairs per treatment condition).

Lastly, we report the blood alcohol concentrations for zebrafish acutely exposed to the reported treatment conditions (0%, 0.125%, 0.25%, 0.5%, and 1.0%). Fish were exposed to a treatment condition for ten-minutes and then blood was extracted for analysis. Figure 5 illustrates the BAC in zebrafish following a ten-minute exposure period.

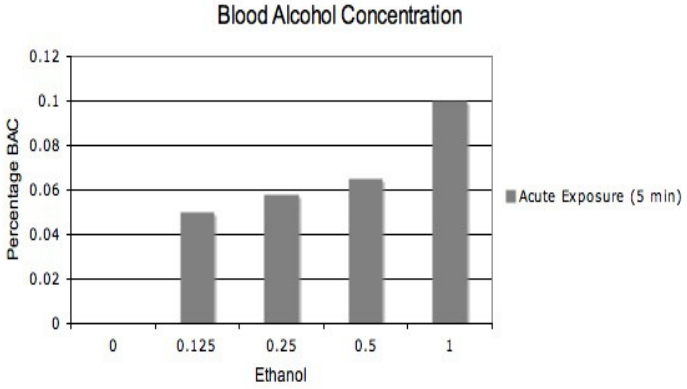

Figure 5. This graph illustrates the blood alcohol concentration that results from a ten-minute exposure to a treatment condition. Mean (±SEM) for BAC is shown ($n = 12$ individuals per treatment group).

Discussion

It has been reported that acute alcohol exposure (0.25%, 0.5%, and 1.0% concentrations) disrupts shoaling behavior (Dlugos & Rabin, 2003; Gerlai, 2003; Gerlai et al., 2000). Previous research from our laboratory did not show shoaling disruption with acute exposure to a 1.0% concentration (Echevarria, Hammack, Pratt, & Hosemann, 2008a). In our laboratory, disrupted shoaling was most evident when followed by exposure to 0.5% and 2.0% ethanol concentrations. Additional testing for immobility and erratic swimming, screening for unwanted side effects, showed that the 2% dose was probably disrupting shoaling by producing global motor and sensory impairments. The 0.5% dose had no effects on immobility or erratic swimming (Echevarria, Hammack, Wooton, Welch, & Rosenblatt, 2008b).
It is well understood that acute alcohol consumption has a modulatory effect on serotonin function. Serotonin is also inversely related to aggression (as cited in McCloskey, Berman, Echevarria, & Coccaro, 2009). The impetus for the current series of experiments was the link between moderate alcohol consumption and aggression. Put another way, does the 0.5% alcohol dose disrupt shoaling by increasing aggression between conspecifics? To test this hypothesis we placed two zebrafish together in an open-field chamber. These pairs had never been housed together and were exposed to one of the following treatment conditions (0%, 0.125%, 0.25%, 0.5%, or 1.0% alcohol concentrations) for a total of ten minutes. As previously reported in Larson et al. (2006) our dependent measures of aggression included: biting, chasing, and retreating. We also measured immobility as a screen for unwanted side effects. Surprisingly, biting was suppressed with exposure to all alcohol concentrations ($p = 0.001$). Overall chase time and overall retreating time was not changed by the alcohol concentrations reported here ($p = 0.44$, $p = 0.53$). However there was a significant change in the *frequency* of chasing and retreating. Exposure to the 0.5% dose resulted in a significant reduction in bouts of chasing and retreating. While there is a significant decrease in instances of these behaviors, our data suggests a dose dependent trend in the average increase in the length of a bout of chasing and retreating. In essence, there are fewer chases and retreats with acute exposure to the 0.5% dose but there is a nearly significant trend in increasing the average length of the bouts. This is interesting in light of the data on overall chase and retreat time, which does not change as a result of treatment exposure.

To better understand the effects of acute alcohol exposure on zebrafish behavior, we thought that it was essential to assess the amount of alcohol that gets into the blood via gill and skin absorption. In a separate series of experiments we looked at alcohol absorption in the zebrafish as a result of acute exposure to alcohol (0%, 0.125%, 0.25%, 0.5%, or 1.0% concentrations). Results show that acute exposure to the 0.5% dose (for 10 minutes) yields a blood alcohol concentration of 0.065%. When comparing the absorption rates it seems as though the 0.5% dose is at the higher end of moderate doses. The doubling of the first three doses (0.125%, 0.25%, 0.5%,) produces a generally linear increase in BAC (0.050%, 0.058%, and 0.065 respectively). The doubling of dose from 0.5% to

1.0% results in a steep increase in BAC (0.065% to near 0.1%) (see Figure 5). Reports from the human literature implicate a 0.06% BAC in the impairment of: reflexes, reasoning, depth perception, distance acuity, and peripheral vision. This BAC is also under the legal limit of 0.08%.

At first glance it may seem as though our data do not support our hypothesis. Clearly the dose range reported here significantly suppressed biting, arguably the primary dependent measure for aggression. Acute alcohol exposure in this dose range also did not change the overall time pairs spent engaged in chasing and retreating. However, the instances, or number of times, a pair engaged in chasing and retreating was significantly reduced in pairs acutely exposed to the 0.5% dose. There is also a very nearly significant trend that reflects an increase in the average length of a chasing and retreating bout. It could be that this 0.5% dose may cause a pair to engage less in chasing and retreating, but once the behavior occurs the pair stays engaged for a longer period of time. This dose, which seems to be on the higher range of moderate, has been reported to significantly increase fin erection / threat display, increase locomotion, and also produce a decrease in the preference for conspecifics (Gerlai, 2003). In light of all of this, we may have an instance where acute alcohol exposure in the dose range reported here produces a fish who's bark is worse than its bite.

References

Barr, C. S., Schwandt, M. L., Newman, T. K., & Higley, J. D. (2004). The use of adolescent nonhuman primates to model human alcohol intake: Neurobiological, genetic, and psychological variables. *Annals of the New York Academy of Sciences, 1021*, 221-233.

Bushman, B. J., & Cooper, H. M. (1990). Effects of alcohol on human aggression: An integrative research review. *Psychological Bulletin, 107*, 341-354.

Dlugos, C. A., & Rabin, R. A. (2003). Ethanol effects on three strains of zebrafish: Model system for genetic investigations. *Pharmacology, Biochemistry and Behavior, 74*, 471-480.

Echevarria, D. J., Hammack, C. M., Pratt, D. W., & Hosemann, J. D. (2008a, November). A novel test battery to assess global drug effects using the zebrafish. *International Journal of Comparative Psychology, 21*, 19-34.

Echevarria, D. J., Hammack, C. M., Wooton, A. J., Welch, A. M., & Rosenblatt, M. P. (2008b, November). The effect of varying doses of alcohol on shoaling behavior in the zebrafish (*Danio rerio*). Poster presented at the Society for Neuroscience conference, Washington, D.C.

Fogarty, J. N., & Vogel-Sprott, M. (2002). Cognitive processes and motor skills differ in sensitivity to alcohol impairment. *Journal of Studies on Alcohol, 63*, 404-411.

Gerlai, R. (2003). Zebra fish: An uncharted behavior genetic model. *Behavioral Genetics, 33*, 461-468.

Gerlai, R., Lahav, M., Guo, S., & Rosenthal, A. (2000). Drinks like a fish: Zebrafish (*Danio rerio)* as a behavioral genetic model to study alcohol effects. *Pharmacology, Biochemistry and Behavior, 67*, 773-782.

Larson, E. T., O'Malley, D. M., & Melloni Jr., R. H. (2006). Aggression and vasotocin are associated with dominant-subordinate relationships in zebrafish. *Behavioral Brain Research, 167,* 94-102.

McBride, W. J., & Li, T. K. (1998). Animal models of alcoholism: Neurobiology of high alcohol-drinking behavior in rodents. *Critical Reviews in Neurobiology, 12*, 339-369.

McCloskey, M. S., Berman, M. E., Echevarria, D. J., & Coccaro, E. F. (2009). Effects of acute alcohol intoxication and paroxetine on aggression in men. *Alcoholism: Clinical and Experimental Research. 33*, 1-10.