Alcohol-induced behavior change in zebrafish models

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Abstract

Zebrafish are at the forefront of neurobiological research and have been gaining popularity as a viable and valid behavioral model in a variety of research applications (e.g., assessing drug induced behavioral changes). This model becomes even more attractive when considering the behavioral changes that follow exposure to compounds that are water-soluble. As such, several studies have implicated oral changes that follow exposure to compounds that are water-soluble. Within this arena there appears to be a common trend across multiple studies. As with many drugs ethanol appears to influence behavior in a dose-dependent manner. In this review, we compare and contrast several studies that measure behavior as a result of alcohol exposure. Appended to this review are pilot data that report zebrafish blood alcohol concentrations as a function of acute exposure.

Keywords: alcohol; behavior; blood alcohol content (BAC); ethanol; shoaling; stress; zebrafish.

Introduction

An increasing number of studies have demonstrated that acute and chronic ethanol exposure affects a variety of zebrafish behaviors. In examining the growing literature available so far, there appears to be a common trend across multiple studies. It appears that ethanol influences behavior in dose-dependent ways, in many cases, reflecting an inverted U-shaped function (i.e., with low to intermediate doses enhancing a variety of behaviors and higher doses generally suppressing them). Here we review several studies in an attempt to summarize, describe and take a closer look at the relationship ethanol has on zebrafish behavior. Table 1 presents a general overview of the behavioral tasks discussed in this review. Tables 2 and 3 present studies that have examined zebrafish behavioral responses to different dosages. Table 2 focuses on studies that have investigated adult behavior (in response to both acute and chronic ethanol exposure) and Table 3 focuses on those that examined larval zebrafish behavior. From a quick glance, one can see that most studies have focused on alcohol concentrations of 0.0%, 0.25%, 0.5% and 1.0% volume percentage. Thus, the following sections summarize the behaviors influenced by this range of doses.

Locomotor behavior

Locomotion has been the most commonly examined endpoint for behavioral analysis with regard to alcohol treatment. Similarly to rodents, humans and other primates (McBride and Li, 1998; Fogarty and Vogel-Sprott, 2002; Barr et al., 2004), alterations in zebrafish motor function from acute alcohol exposure have been described as an inverted U-shaped function. Low to intermediate doses result in hyperactivity compared to higher doses, which result in hypoactivity. For example, two studies (Gerlai et al., 2000; Gerlai, 2003) examined the number of crossings between four segments in an observation tank as the measure of locomotion. In both studies, acute exposure to 0.25 and 0.50 v/v% ethanol concentration resulted in significant increases in locomotor activity compared to controls (0.0%), both after 1 min and after 10 min post-exposure. However, at the 1.0% concentration level, activity fell significantly below that of the control group, an effect that diminished by the tenth minute. This suggests a potential sedative effect with exposure to the higher levels of alcohol. These results are supported, in part, with research done by another group (Lockwood et al., 2004), who examined the responses of larval zebrafish to alcohol. Their results also demonstrate a dramatic dose-response curve, although with one major difference. Significant hyper- and hypoactivity occurred at higher concentrations than those presented in previous reports (Gerlai et al., 2000; Gerlai, 2003). More specifically, hyperactivity was not apparent until exposure to the 1.0% alcohol concentration. Furthermore, mean locomotor activity did not characteristically decrease until the 3.0% concentration (at which point, this was probably due to unwanted side effects, as this treatment was not significantly different from control groups), and hypoactivity was not evident until concentrations as high as 4.0%. This difference could be attributed to the use of more hardy larvae compared with adults, or to the difference in exposure durations and procedure. Lockwood et al. (2004) examined behavior from the start of exposure, over the course of only 20 min. By contrast, Gerlai and colleagues (Gerlai et al., 2000; Gerlai, 2003) exposed fish to alcohol for 60 min, and then recorded behavior for an additional 10 min in the same conditions.
Table 1  Summary of behavior tasks discussed in the text.

<table>
<thead>
<tr>
<th>Behavioral tests</th>
<th>Generally used to examine</th>
<th>General description</th>
<th>Potential measures</th>
<th>References</th>
</tr>
</thead>
</table>
| Open field                | Activity/locomotor behavior                                                              | Present subject(s) with an open, homogeneous environment, commonly void of any structural components                                                                                                           | Ambulation/rate of locomotion/swim speed (typically the number of squares traversed over a grid)  
Percent of total activity  
Spatial distribution  
Other forms of specific locomotor behavior (e.g., passive, creeping or drifting movement)                                                                 | Gerlai et al., 2000; Gerlai, 2003; Lockwood et al., 2004; Gerlai et al., 2006; Fernandes and Gerlai, 2009 |
| Mirror exposure           | Aggression                                                                               | Animal is confronted with its mirror image. Can be straight on or incline mirror                                                                                                                                     | Time spent closest to mirror  
Aggressive displays  
Biting, chasing/retreating frequencies and/or durations  
Measures of dominant/subordinate relationships                                                                                                                                               | Gerlai et al., 2000; Gerlai, 2003; Larson et al., 2006; Spence et al., 2008; Echevarria et al., 2010 |
| Shoaling                  | Propensity to approach or spend time with conspecifics  
Group shoaling dynamics  
A group of fish is typically placed in an open-field type environment and disruptions to social cohesion are examined | Presents subject(s) with a group of conspecifics behind a partition at one end of a tank  
A group of fish is typically placed in an open-field type environment and disruptions to social cohesion are examined                                                                 | Propensity for shoaling: percent of time spent closest to conspecifics, distance from conspecifics  
Shoaling dynamics: inter-fish distance, nearest neighbor distance, mean area occupied by entire group | Gerlai et al., 2000; Dlugos and Rabin, 2003; Fernandes and Gerlai, 2009 |
| Predator exposure         | Anxiety/fear/alarm response                                                              | Presents subject(s) a predator or predator model. Could be in open-field type environment  
Predator/model could be behind partition in the same environment, in a separate environment with visible access or overhead | Distance from stimulus  
Duration spent close to stimulus  
Predator inspection, jumping, freezing behaviors | Gerlai et al., 2000; Gerlai et al., 2006; Bass and Gerlai, 2008; Speedie and Gerlai, 2008 Fernandes and Gerlai, 2009 |

Note: A summary of many of the tasks presented in this review. Please note that this is not an exhaustive list of tasks that have been used in behavioral studies related to alcohol nor are the measures included here an exhaustive list of those available for each task. This is simply a summary of how the tasks reported in this review have been utilized.
Table 2  Alcohol exposure studies with adult zebrafish in acute and chronic treatment conditions.

<table>
<thead>
<tr>
<th>Behaviors investigated</th>
<th>Specific measures</th>
<th>Ethanol exposure concentration (acute, % volume)</th>
<th>Ethanol exposure concentration (chronic, % volume)</th>
<th>During or after exposure?</th>
<th>Exposure time</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.0</td>
<td>0.125</td>
<td>0.25</td>
<td>0.30</td>
<td>1.0</td>
</tr>
<tr>
<td>Startle reaction group</td>
<td>Number of squares traversed, post-startle stimulus</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Swimming/shoaling behavior</td>
<td>Nearest neighbor distance, mean area occupied</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggression</td>
<td>Biting, chasing frequency, chasing duration, retreating frequency, retreating duration</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Immobility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locomotor activity</td>
<td>Ambulation and horizontal location</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Aggression (inclined mirror task)</td>
<td>Spatial distribution along incline mirror and aggressive display</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social preference</td>
<td>% Time spent near stimulus group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antipredator behavior</td>
<td>Jumping frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light/dark preference</td>
<td>Duration spent in dark</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigment response</td>
<td>Color saturation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locomotor activity</td>
<td>Ambulation and horizontal location</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Aggression (inclined mirror task)</td>
<td>Spatial distribution along incline mirror and aggressive display</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Social preference</td>
<td>% Time spent near stimulus group</td>
<td></td>
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<tr>
<td>Pigment response</td>
<td>Color saturation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locomotor activity</td>
<td>Ambulation and horizontal location</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Aggression (inclined mirror task)</td>
<td>Spatial distribution along incline mirror and aggressive display</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social preference</td>
<td>% Time spent near stimulus group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigment response</td>
<td>Color saturation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locomotor activity</td>
<td>Path length</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Antipredator behavior</td>
<td>Jumping; distance from predator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habituation to novel environment</td>
<td>Time spent at top of tank, frequency of transitions to top, frequency of erratic movement, frequency and duration of freezing</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Note: The specific measures listed for each number in column two corresponds to the same number listed in column one. The column labeled ‘During or after exposure?’ is with regards to when the behavior reported for the study was sampled in relationship to when fish were exposed (e.g., ‘during’ indicates that the behavioral results reported were collected during the reported exposure treatment; ‘after’ indicates behavioral results were reported from data collected after initial exposure treatment). Echevarria et al. (2010) examined locomotor and shoaling behaviors ‘during’ the 60-min exposure treatment, but ‘circling’ was examined ‘following’ exposure. Furthermore, ‘shoaling’ was restricted to the 1.0% treatment condition, and exposure was only 30 min.
Table 3. Alcohol exposure studies with larval zebrafish in acute treatment conditions.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Larval age</th>
<th>Exposure time</th>
<th>Exposure concentration (% volume)</th>
<th>During or after exposure?</th>
<th>Other measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lockwood et al. (2004)</td>
<td>2 dpf</td>
<td>6-7 months later</td>
<td>0.0, 0.25, 0.50, 1.0, 1.5, 2.0</td>
<td>√</td>
<td>Locomotion (swim speed), Whole-body ethanol absorption</td>
</tr>
<tr>
<td>Fernandes and Gerlai (2009)</td>
<td>24 hpf</td>
<td>2 h</td>
<td>0.0, 0.25, 0.50, 1.0</td>
<td>√</td>
<td>Thigmotaxis, Whole-body ethanol absorption</td>
</tr>
<tr>
<td>Carvan et al. (2004)</td>
<td>Up to 6 dpf</td>
<td>After</td>
<td>0.0, 0.25, 0.50, 1.0, 10</td>
<td>√</td>
<td>Social preference, Cell death</td>
</tr>
</tbody>
</table>

Note: The column labeled ‘During or after exposure?’ is with regards to when the behaviors reported for the study was sampled in relationship to when fish were exposed; dpf = days post-fertilization; hpf = hours post-fertilization. The concentrations are listed here in increasing order and should not be confused with the column headings (% volume) above.

The effects seen in locomotor activity also appear to be dependent upon the strain of zebrafish. Lockwood et al. (2004) demonstrated that although both AB and WIK larval strains exhibited hyperactivity at 1.5% alcohol concentrations, the WIK strain not only had higher basal levels of activity but also the effect of alcohol on activity (swim speed) was much less than that exhibited by the AB strain (mean difference in swim speed between basal and 1.5% treatment condition: WIK=1 mm/s, AB=3.7 mm/s). This effect was not examined over varying dose levels. It would be interesting to see if the dose-dependent response was consistent for both strains, even if the degree of the effect differed.

It is important to note two additional studies that did not demonstrate the typical inverted U-shaped dose-response curve for locomotor measures. In the first study, Fernandes and Gerlai (2009) found that acute alcohol exposure in larval zebrafish, 24 hours post-fertilization, did not result in sustained hyperactivity by 6–7 months of age. This suggests that hyperactivity is not a permanent side effect of alcohol exposure during development (at least at concentrations between 0.0% and 1.0%). In the second study, Gerlai et al. (2006) used identical methods as those in Gerlai et al. (2000), and yet typical hypoactivity was not demonstrated at the 1.0% acute dose treatment condition. Instead, hyperactivity increased, nearly linearly, across increasing exposure concentrations. Although further research is needed to elucidate the correct alternative, the authors suggest a couple of potential explanations. It is possible that this again reflects a strain-dependent response, because the more recent study was done using long-fin wild-type strain instead of the previously utilized California outbred stock. Furthermore, they acknowledge a change in measures from the manual counting of segment-crossings to a more precise, automated, continuous tracking system, which calculated path length. Based on the new findings, Gerlai et al. (2006) suggest that the effects of high acute doses could be more complex than previously thought, and that there might not be a universal sedation effect at higher doses, such as previously thought.

Gerlai et al. (2006) also examined the effects of acute alcohol treatment and acute alcohol treatment after chronic alcohol pre-exposure (acute X chronic interaction) effects on behavior in adult zebrafish. This was a 4×2 experimental design with four acute and two chronic ethanol exposure conditions. Zebrafish in chronic conditions were exposed to either freshwater or to 0.25% ethanol concentrations for 2 weeks, and then behaviorally tested in a range of acute alcohol exposure conditions. Zebrafish in chronic conditions were exposed to either freshwater or to 0.25% ethanol concentrations for 2 weeks, and then behaviorally tested in a range of acute alcohol exposure conditions (0.00%, 0.25%, 0.50% and 1.0%). This produced a total of the following eight treatment groups, as presented by the authors: C0.00–A0.00, C0.00–A0.25, C0.00–A0.50, C0.00–A1.00, C0.25–A0.00, C0.25–A0.25, C0.25–A0.50 and C0.25–A1.00, where C indicates chronic treatment and A indicates acute treatment (Gerlai et al., 2006). Therefore, the behaviors examined were a result of the combined effect of exposure to both chronic and acute treatment conditions. There was no significant effect of chronic treatment alone; however, there was a significant interaction between acute and chronic treatments [F(3, 98)=2.74, p<0.05, p=7]. Zebrafish exhibited hyperactivity in both 0.5% and 1.0% acute treatment conditions.
conditions when previously treated chronically for 2 weeks (i.e., groups C0.25–A0.50 and C0.25–A1.00). Interestingly, when these groups were placed back in the 0.25% acute condition, their activity returned to basal levels (i.e., no different from the control, never-exposed, freshwater condition). The authors point out that chronic exposure appeared to reduce the hyperactivity associated with the acute ethanol treatment. They further suggest that this could be a result of adaption to ethanol. This study did not, however, examine more than one chronic ethanol treatment condition. More research is needed to examine how different chronic ethanol exposure treatments influence activity.

Aggression

Alcohol is known to induce aggressive behavior in humans [for a review, see (Bushman and Cooper, 1990)]. Zebrafish have been shown to exhibit aggressive behaviors, including biting, chasing and aggressive displays (Gerlai et al., 2000; Larson et al., 2006; Spence et al., 2008; Echevarria et al., 2010), furthering their utility as a model organism for examining the effects of alcohol. Similar to locomotor behavior, alcohol appears to influence aggressive behavior in a dose-dependent manner, representing an inverted U-shaped function.

Gerlai and colleagues (Gerlai et al., 2000; Gerlai, 2003) measured aggression using a mirror task, in which the mirror was placed at an incline to the back wall of a tank. An increase in the duration of aggressive displays towards the mirror was associated with an increase in the amount of time spent closest to the mirror, supporting the use of relative duration of time near a mirror as an indication of aggressive behavior (i.e., as opposed to the alternative possibility that it was an indication of shoaling propensity). They found that intermediate alcohol treatments resulted in a general increase in aggression. More specifically, in analyzing time spent nearest the mirror, there was a significant increase in the first minute following exposure in the 0.25% treatment condition (compared to other doses). By minute 10, both the 0.25% and 0.5% conditions exhibited significantly higher amounts of time spent closest to the mirror, compared to 0.0% and 1.0% treatments. Similarly, there was a significant alcohol effect for the measure of aggressive display duration, which exhibited the characteristic inverted U-shaped dose curve regardless of time, i.e., the 0.25% condition produced the largest increase in aggressive display behavior, an effect that fell below control levels in the 1.0% condition.

Echevarria et al. (2010) examined the effects of alcohol on aggressive interactions between pairs of zebrafish (over concentrations of 0.0%, 0.125%, 0.25%, 0.5% and 1.0%). Surprisingly, however, they found that the average frequency of biting decreased significantly for each dose treatment, compared with controls [F(4, 75)=5.49, p<0.01]. Furthermore, there was no difference in the average duration of chases or retreats across the 10-min exposure period, for any dose condition. There was, however, a significant decrease in the frequency of chasing and retreating behavior in the 0.5% concentration level (compared with controls), which upon closer inspection, was potentially explained by an increase in the average duration of each chase and retreat bout. This increase just narrowly missed significance for chases [F(4, 75)=2.34, p=0.06] and was at the significance cut-off level for retreats [F(4, 75)=2.42, p=0.05]. This relationship requires further examination, possibly including a longer exposure time, but the data suggests that alcohol can increase the intensity of each aggressive bout.

Schooling/shoaling social behavior

The zebrafish are a shoaling species. These social animals naturally aggregate in multimember groups, demonstrating a preference for conspecifics. Shoaling groups function to decrease predation risk and optimize resource acquisition [as summarized by (Wright et al., 2003)], and social experiences can influence antipredator behavior, foraging and behavioral development in individuals (Moretz et al., 2007). Therefore, disruptions to this behavior can have significant consequences for both the individual and the group.

Research has shown that alcohol disrupts shoaling behavior. Instead of the inverse dose-response found in motor behavior, shoaling disruption appears to follow a positive, nearly linear pattern in response to increasing doses. Dlugos and Rabin (2003) quantified the influence of acute 0.0%, 0.25%, 0.5% and 1.0% ethanol treatments on adult zebrafish shoaling, measured via nearest neighbor distances and the mean area the group occupied. Alcohol increased nearest neighbor distance and mean area occupied in each treatment condition, compared with controls, for two out of the three strains examined (wild type: WT; long-fin striped: LFS). The authors did not report if there was significance found between treatment conditions, only for each treatment compared with controls. However, from the figures presented, it appears that the highest concentrations disrupted shoaling more so than low and intermediate concentrations (in both measures used, although significance is unknown), appearing as a positive linear trend in disruption with increasing doses. It is important to note that this relationship was found to be strain-dependent. Shoaling was not found to be disrupted even in the highest concentrations (1.0%) for the blue long-fin strain (BLF). In the same study, Dlugos and Rabin also examined the effects of chronic exposure (for 1 and 2 weeks) at the 0.5% concentration level. Shoaling was disrupted in the WT strain, exhibited by a 29% increase in mean nearest-fish distance after 1 week of exposure, and a 39% increase by week 2 (both compared with baseline levels). However, again, chronic exposure was strain-dependent; this time shoaling was not disrupted in the LFS strain (the BLF strain was not tested).

A different approach to examining shoaling/social behavior has also been examined, the results of which have also indicated disruptive effects of alcohol treatments. Noting that zebrafish tend to exhibit a preference for conspecifics, Gerlai et al. (2000) investigated how alcohol influences this preference (measured as the percentage of time spent close to the side of the tank nearest to conspecifics) across doses (0.0%, 0.25%, 0.5% and 1.0%). There was a dose-dependent, nearly linear,
decrease in preference (for an entire group of five treatment fish) with increasing doses, suggesting a disruption in shoaling behavior. The authors report that higher concentrations of alcohol led to ‘...a more scattered, distributed spatial location of the experimental fish...’ (p. 778), which is consistent with the results reported by Dlugos and Rabin (2003). Fernandes and Gerlai (2009) also examined alcohol effects on social preference, by looking at how ethanol exposure during development influences social preference as adults. The control (0.0%) group of adult fish that were not previously exposed to alcohol (at 24 hpf) showed an expected dramatic decrease in distance from conspecific stimulus when compared with no stimulus, demonstrating a preference for conspecifics without having had any alcohol treatment during development. However, this preference linearly decreased with increasing alcohol concentration exposure, such that the highest dose group (1.0%) exhibited the smallest reduction in distance from conspecifics (i.e., the least preference for conspecifics), such that it was not even significantly reduced compared with when there was no stimulus. This means that the effects of alcohol administered during development still disrupted typical social behavior 6–7 months post-treatment, and did so in a dose-dependent manner. This differs from the motor behavior described earlier, in which hyperactivity, characteristic of low to intermediate doses of alcohol exposure, was not found to persist into adulthood (Fernandes and Gerlai, 2009).

Anxiety and fear

Predator models have become an increasingly accepted tool for measuring fear responses in many species, including zebrafish (Bass and Gerlai, 2008; Speedie and Gerlai, 2008). This type of paradigm can aid in furthering our understanding of anxiety and phobias in humans. Research has shown that alcohol influences the characteristic behavioral response of adult zebrafish when exposed to predators, in which case alcohol could be acting on anxiety, perceptual or motor mechanisms (Gerlai et al., 2000). Gerlai et al. (2000) found that alcohol exposure modified the predator response (i.e., the frequency of jumping behavior in response to predator exposure) and did so in an inverted U-shaped manner over increasing doses (0.0%, 0.25%, 0.5% and 1.0%). The 0.25% concentration level exhibited the highest response (highest jumping frequency), which was nearly extinguished at 1.0% concentration levels (significantly less than control levels).

The study (already described in more detail under locomotor behavior) conducted by Gerlai et al. (2006), examining acute alcohol treatment and acute X chronic interaction effects on behavior, also examined predator responses under these conditions. Jumping behavior was again one of the behavioral endpoints examined. Typical jumping responses, associated with predator exposure, were significantly more frequent at lower acute doses (groups C0.00–A0.00 and C0.00–A0.25), but became non-significant with acute exposures of 0.5% and 1.0% concentrations (groups C0.00–A0.50 and C0.00–A1.00) (i.e., there was no increase in jumping associated with predator exposure, compared with non-predator exposure times, at these concentrations). A similar pattern of jumping behavior was found in the chronic C0.25 groups, in which higher acute concentrations (groups C0.25–A0.50 and C0.25–A1.00) resulted in a decrease in jumping behavior.

The chronic ethanol exposure itself did not have a significant effect on the jumping behavior; although, it is important to note that without any subsequent acute exposure, chronic exposure alone (C0.25–A0.00) led to an increase in jumping behavior in response to the predator, more so than at any other treatment condition (after 1 min of behavioral recording, postexposure). For jumping behavior, higher acute doses following chronic ethanol treatment, once again, resulted in extinguishing the typical fear response associated with predator exposure.

This effect was supported by a second measure of predator response used in the same study (Gerlai et al., 2006). The second measure examined predator avoidance behavior (i.e., distance from predator when the predator stimulus was presented). Control groups (C0.00–A0.00) respond to predators by moving away from the side of the tank where the predator is, significantly more so than when there is no predator and the 0.25% acute treatment groups (C0.00–A0.25) also show this response. However, as with jumping behavior, this typical response was diminished in the 0.5% and 1.0% acute treatment groups (i.e., C0.00–A0.50 and C0.00–A1.00). As seen with jumping behavior, chronic C0.25 groups demonstrated results comparable to those with C0.00 groups. The major difference in the distance-from-stimulus measure was in the chronic followed by 0.5% acute treatment condition (C0.25–A0.50). Unlike the response in the C0.00–A0.50 groups (without chronic ethanol exposure), fish in the C0.25–A0.50 (with chronic ethanol exposure) did continue to respond to predators by moving further away. The authors suggest that the chronic ethanol exposure resulted in an attenuation of the effect seen in the acute-only exposure. Furthermore, they suggest that this might indicate an adaption effect due to long-term exposure, similar to that described earlier for motor behavior in this study. In summary, the two studies conducted by Gerlai and colleagues (Gerlai et al., 2000, 2006) provides evidence for anxiolytic-like effects of ethanol in zebrafish at higher concentrations, and evidence that chronic exposure in zebrafish has the potential to result in adaptation to alcohol.

Predator response behavior after alcohol treatments was also examined by Fernandes and Gerlai (2009). They found that although predator exposure itself influenced the behavior of adult fish (i.e., increased the distance of groups from the predator stimulus), there were no changes reflected across different doses when alcohol had been previously administered in the larval stage. Thus, acute exposure to alcohol during development does not appear to result in anxiolytic effects in adulthood (in doses between 0.0% and 1.0%).

Wong et al. (2010) also examined the anxiolytic effects of alcohol in zebrafish behavior. Instead of a predator model, they tested responses to a novel tank, for which they first examined habituation to the novel tank under control conditions (over 6 min). In the acute treatment condition, a 0.3% ethanol concentration was administered for 5 min and behavior was recorded (from a side view) for 6 min,
following treatment. They found that towards the end of the observation period (5–6 min), acute exposure resulted in an increased number of transitions to the top of the tank, and an increase in the amount of time spent at the top half of the tank (although erratic movement and freezing behavior remain unchanged). High stress and anxiety have generally been shown to be correlated with reduced erratic movement and exploration (e.g., fewer transitions to the top of a tank, more freezing behaviors) in a novel environment. Therefore, results by Wong et al. (2010) indicate an anxiolytic effect with acute alcohol treatment. Other treatment doses were not examined in this study, but these results are consistent with the anxiolytic effects of other intermediate acute dose levels (0.25% or 0.50%). Additionally, Wong and colleagues (2010) found the same effects from a 2-week chronic exposure treatment in a 0.20% concentration (i.e., an increased number of transitions to the top of the tank, an increase in the amount of time spent at the top half of the tank, and no change in erratic movement or freezing).

Dlugos and Rabin (2003) examined the startle response of individual fish to a glass bead lowered in front of them. With a grid placed under the behavioral apparatus, they measured the number of 9 cm² blocks traversed by the fish as it moved away from the bead. Generally, acute exposure resulted in a decrease in startle response (i.e., a decrease in the number of squares traversed after exposure to the stimulus). Similar to behaviors previously discussed (locomotion and shoaling), the effects of alcohol on the startle response was strain-dependent. The WT strain only showed a significant decrease in startle response with a dose of 0.5%, and once again the BLF strain showed no effect. The LFS strain, however, exhibited a significant decrease in startle response at all ethanol concentration levels, compared with controls (0.25%, 0.5% and 1.0% compared with 0.0%). The mean number of squares traversed for each dose, in increasing dosage-order (0.0 % and 1.0 % compared with 0.0 %). The mean number of squares traversed for each dose, in increasing dosage-order (0.0 % and 1.0 % compared with 0.0 %).

Alcohol absorption

Alcohol is water-soluble and can be readily added to the zebrafish aquarium. The paradigms listed here usually measure simple behaviors (the subject undergoes little to no training) and are relatively easy-to-use. Very often the apparatus is based on a modified standard aquarium available from pet stores, which makes replication across laboratories a simple feat. As a result, researchers in this field have the luxury of choosing from several relatively inexpensive ways to examine the behavioral effects of alcohol. However, little is really known about how much alcohol is actually getting into the general circulation of the zebrafish. This is especially apparent when considering the adult zebrafish. When considering zebrafish fry, Lockwood et al. (2004) examined whole organism levels of internal ethanol concentration in 7 dpf zebrafish fry, exposed to 1.5% (for 1, 10 and 20 min) and 3.0% (after 20 min) concentration levels (i.e., the levels that caused the highest and lowest hyperactivity and hypoactivity, respectively). After exposure, subjects were immediately euthanized with tricaine and rinsed to remove ethanol from the skin. Results demonstrated a positive linear function over time in the 1.5% concentration condition. The internal concentrations were found at 0.04%, 0.08% and 0.12% (w/v) over 1, 10 and 20 min, respectively. Exposure for 20 min in the 3% condition resulted in an even higher internal level of 0.33% (w/v).

Dlugos and Rabin (2003) examined how much of a 0.5% (v/v) alcohol bath was getting into the adult zebrafish brain at various time points across a 24-h exposure time. This effect was examined in three different strains (WT, LFS and BLF). Within 15 min of exposure, significant alcohol levels were already present in the brain for all three strains. Although specific levels are not reported, the brain ethanol content was between 1 and 2 µg/mg wet weight. The uptake appeared to quickly reach a saturation level (just under 2 µg/mg wet weight for LFS strain, and between 2 and 3 µg/mg wet weight for the WT and BLF strains) that was consistent across the 24-h period. The authors report that at this level the brain alcohol levels were approximately 90% of the tank alcohol level. There were no differences over time, across strain, nor any interaction effects. It is important to note that although
several alcohol-induced behavioral changes, discussed earlier, exhibited strain-dependent behavioral responses (from this study) the brain ethanol content was no different between strains.

In light of this, in a separate series of experiments we measured blood alcohol concentrations (BACs), using a spectrophotometer, in zebrafish after acute exposure to the following doses: 0.0%, 0.0625%, 0.125%, 0.25%, 0.5%, 0.75% and 1.0% (pilot data shown as Figure 1). We chose these doses in an attempt to define a range that encompasses what is generally reported in the literature. To quantify how much ethanol was absorbed into the zebrafish, the project used the EnzyChrom Ethanol Assay Kit (ECET-100) from BioAssay Systems (Hayward, CA, USA). Twelve fish per treatment group (dose) were exposed to a condition for 5, 15 or 30 min. Immediately afterwards, fish were anesthetized with MS-222 (Tricane) and blood was extracted for processing. It is important to note that 12 fish yielded one sample with approximately 50–70 ml of blood, or one data point. The pilot data presented in Figure 1 is preliminary and the experiment is ongoing.

Interestingly, when acute exposure is for 5 or 15 min all doses (0.0625%, 0.125%, 0.25%, 0.5%, 0.75% and 1.0%) follow a very similar pattern of absorption, with the smallest and highest doses for both time points being nearly identical. This stepwise pattern of alcohol uptake is expected and takes place between the BAC levels of 0.04% and 0.1%. When acute exposure is for 30 min, doses 0.0625%, 0.125%, 0.25% and 0.5% result in a similar absorption pattern to the shorter exposure times mentioned above, and result in BAC levels ranging from 0.05% to 0.09%. However, the larger doses (0.75% and 1.0%) yield a higher BAC level after 30 min of acute exposure. Specifically, after 30 min the 0.75% dose results in a BAC of 0.168% (the highest BAC of any dose or time point); this is in contrast to the 1.0% dose, which after 30 min results in a slightly smaller (but still large) BAC of 0.117%. When one consults the literature (see Tables 1 and 2 for a summary), it is rather amazing that, to date, no one has looked at the effects of 0.75% alcohol on adult zebrafish behavior. It is possible that this drastic increase in BAC could alter a behavior that has not been assessed as of yet.

The ever-growing literature on zebrafish behavior seems to show that we are on the precipice of seeing this animal model becoming a rival for the title of ‘the lab standard’. The ongoing dissemination and sharing of results will help fill in the missing pieces of not only this puzzle but many other puzzles also. Regardless of the research questions being asked, reported results continue to support the validity and viability of the zebrafish as an animal model of behavior.

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References


Figure 1 Pilot data for blood alcohol concentrations in adult zebrafish after acute exposure (30, 15 or 5 min) to alcohol concentrations of 0.0625%, 0.125%, 0.25%, 0.50%, 0.75% and 1.0%. Each data point represents the blood extraction from 12 individual fish.