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Behavioural responses to novelty or to a predator stimulus are not altered in adult zebrafish by early embryonic alcohol exposure

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Abstract

Background—Fetal Alcohol Spectrum Disorders (FASD) may vary in symptoms and severity. In the milder and more prevalent forms of the disease, behavioural abnormalities may include impaired social behaviour, e.g. difficulty interpreting social cues. FASD patients remain often undiagnosed due to lack of biomarkers, and treatment is unavailable because the mechanisms of the disease are not yet understood. Animal models have been proposed to facilitate addressing these problems. More recently, short exposure of the zebrafish embryo to low concentrations of alcohol was shown to lead to significant and lasting impairment of behaviour in response to social stimuli. The impairment may be the result of abnormal social behaviour or altered fear/anxiety. The goal of the current study was to investigate the latter.

Methods—Here, we employed the alcohol exposure regimen used previously (exposure of 24th hour post-fertilization embryos to 0.00, 0.25, 0.50, 0.75 or 1.00 vol/vol % alcohol for 2 hours), allowed the fish to reach adulthood, and measured the behavioural responses of these adults to a novel tank (anxiety related behaviours) as well as to an animated image of a sympatric predator of zebrafish (fear related behaviours).

Results—We found behavioural responses of embryonic alcohol exposed adult fish to remain statistically indistinguishable from those of controls, suggesting unaltered anxiety and fear in the embryonic alcohol treated fish.

Conclusions—Given that motor and perceptual function was previously shown to be also unaltered in the adults after embryonic alcohol exposure, our current results suggest that the impaired response of these fish to social stimuli may be the result of abnormal social behaviour.

Keywords

anxiety; ethanol; fear; fetal alcohol exposure; foetal alcohol exposure; social behaviour; zebrafish

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INTRODUCTION

Children exposed in utero to alcohol (ethanol, ethyl alcohol or EtOH) may exhibit a range of abnormalities (Jones & Smith, 1973, Streissguth et al., 1978). The diagnostic umbrella term, fetal alcohol spectrum disorder (FASD) includes gross anatomical abnormalities at the most severe end of the spectrum, and only mild behavioural alterations at the least severe end (Nash et al., 2008). The milder forms of the disease are more prevalent (Roozen et al., 2016; May et al., 2009; May & Gossage, 2001). Children at this end of the spectrum may display a variety of alterations in social behaviour, including social responding, reading social cues, interpreting the mental states and emotions of others, empathy, perspective taking, social problem solving, and aggression without any obvious anatomical abnormalities (Nash et al 2006; Stevens et al., 2012; 2015; Greenbaum et al., 2009). Rodent models of FASD have recapitulated some of these abnormalities, and, for example, found increased aggression (Hamilton et al., 2014) and impaired social recognition in alcohol treated rats (Varlinskaya & Mooney, 2004). While most animal models of FASD utilize rodents, recently the zebrafish has also been demonstrated to be an appropriate laboratory species for the analysis of the physiological and anatomical (Bilotta et al., 2004; Sadrian et al., 2014) as well as the behavioural and neurochemical effects of embryonic alcohol exposure (Fernandes & Gerlai, 2009; Buske & Gerlai, 2011; Mahabir et al, 2014).

Early embryonic alcohol treatment in zebrafish has been found to consistently produce a marked change in responding to social stimuli when low concentrations of alcohol were used for short durations of time (Fernandes & Gerlai 2009; Buske & Gerlai, 2011). Fish exposed to alcohol for only 2 hours at 24 hours post-fertilization (hpf) and tested in adulthood exhibited decreased social cohesion (increased inter-individual distance) when swimming in groups (Buske & Gerlai 2011), and increased distance to animated conspecific images when individually placed in a tank at 6 months (Fernandes & Gerlai, 2009) or two years of age (Fernandes et al., 2015a). These results suggest that the acute embryonic alcohol treatment has persistent long lasting adverse effects and impairs the ability of adult zebrafish to respond to social stimuli, which mimics some of the social impairments found in human studies.

Although the analysis of the possible factors and mechanisms underlying the observed abnormal response of embryonic alcohol exposed zebrafish to social stimuli is only beginning, impairment of motor function and/or perception has already been excluded (Fernandes & Gerlai, 2009; Buske & Gerlai, 2011). Another important factor, however, has not been explored. It is possible that the reported impaired responses to social stimuli were the result of altered fear or anxiety. Shoaling, i.e. group forming, is a typical feature of the zebrafish (Miller & Gerlai, 2007). Its main adaptive function is to minimize the risk of predation (Suriyampola et al., 2016; Foster & Trehern, 1981). For example, under aversive conditions, shoal cohesion increases (distance among shoal members decreases) in zebrafish (Speedie & Gerlai, 2008; Miller & Gerlai, 2007). Altered fear and or anxiety thus may modify shoaling responses too. It is plausible that in previous studies, embryonic alcohol exposure affected the mechanisms of fear or anxiety, and this led to the observed impairment of social stimulus induced responses (Fernandes & Gerlai, 2009). Alternatively, it is also possible that fear or anxiety remained unaltered, and the embryonic alcohol treatment

affected social behaviour itself, and, for example, altered the motivation to shoal, or altered the ability of zebrafish to recognize other zebrafish as shoal-mates.

The current study was designed to investigate whether the previously discovered embryonic alcohol-induced social impairments are due to altered anxiety or fear in zebrafish. Anxiety and fear are related phenomena and it is debated whether they are phenotypically and/or mechanistically distinct (Perusini & Fanselow, 2015). Here, we adopt an operational definition (for further discussion of this topic in the context of zebrafish research, see Gerlai et al, 2009). We define anxiety as the behavioural responses induced by aversive stimuli that are diffuse and whose onset and offset is not clearly defined. Exposure to a novel environment is often regarded as anxiety inducing (Stewart et al., 2012; Bencan et al., 2009). Fear, on the other hand, represents the behavioural responses induced by a clearly present aversive stimulus whose onset and offset is well defined, and whose period of presentation is short (seconds to a minute). To examine any potential alcohol-induced differences in anxiety levels, control and embryonic alcohol-exposed fish were placed in a novel tank. Numerous behavioural responses have been utilized to evaluate anxiety. These include, for example, erratic movement, vertical exploration of the tank, distance of the fish to bottom of the tank, and overall activity level (Gerlai, 2010; Maximino et al 2010; Stewart et al., 2012). Previously, using a novel tank task after chronic embryonic alcohol exposure (8 days), Baiamonte et al. (2016) found that embryonic alcohol exposure led to a decrease in anxiety related behaviours in adult zebrafish. It is not known, however, how an acute (2hr long) treatment with low concentrations of alcohol during embryonic development would affect anxiety in adult zebrafish.

We also examined fear, by comparing the responses of embryonic alcohol-exposed and control fish to an animated image of a sympatric predator of zebrafish. Zebrafish have been shown to respond robustly to moving predator images displayed on a computer screen (Ahmed et al., 2011; Ahmed et al., 2012). Zebrafish may react to predator images in a variety of ways (Gerlai, 2010) that may be classified as either active avoidance (panic, fleeing/escape, and erratic movements) (Egan et al, 2009; Bass & Gerlai, 2008; Ladu et al, 2015) or passive avoidance (remaining still, or moving more slowly) (Blaser et al, 2010). If fear is altered due to the embryonic alcohol treatment, numerous active and/or passive avoidance reactions may be modified in the affected fish.

Last, because fear and anxiety have not been investigated in the particular FASD zebrafish model we aim to study, and because these behavioural responses are highly context dependent (Gerlai, 2010), we decided to manipulate an important physical parameter known to influence these responses, the size of the test tank. Antipredatory behaviour is dependent upon how far the predator is from the prey and how much room there may be for the prey to escape (Magurran & Girling, 1986). To compare the effect of the size (length) of the experimental tanks, we tested our embryonic alcohol exposed fish in a 100 cm long as well as in a 50 cm long tank. These tank sizes were employed in the past by separate independent studies (Ahmed et al., 2011; Ahmed et al., 2012) for the analysis of fear and anxiety related behavioural responses of zebrafish, but never in a systematic randomized manner in the same study.

MATERIALS AND METHODS

Embryonic alcohol Treatment

We chose the zebrafish as our model organism for numerous reasons. Authors of zebrafish alcohol studies, including those working on FASD models (Bilotta et al., 2004; Fernandes & Gerlai, 2009; Buske & Gerlai, 2011; Sadrian et al., 2014; Mahabir et al, 2014) emphasize that the zebrafish represents an optimal compromise between system complexity and practical simplicity. It is a reductionist model with numerous advantages, which include evolutionary conserved features of its biology and genetics, the prolific nature of this species, the low cost of its maintenance, its fast development, the fact it develops externally and without the influence of maternal physiology or behaviour, and the fact that alcohol may be precisely delivered at predetermined developmental stages represent advantages over other model species. The AB strain was chosen as the focus of the current study because this was the strain numerous prior FASD studies utilized (Fernandes & Gerlai, 2009; Buske & Gerlai, 2011; Mahabir et al, 2014). (Adult zebrafish of the AB strain were bred in-house (University of Toronto Mississauga Vivarium) to obtain fertilized eggs. Eggs were collected shortly after fertilization, and were maintained in system water (deionized and sterilized water supplemented with 60mg/l Instant Ocean Sea Salt, Big Al's Pet Store, Mississauga, ON, Canada) in an incubator at a constant temperature of 28 °C. At 24h post-fertilization, eggs were placed in a solution containing either 0.00, 0.25, 0.50, 0.75, or 1.00% vol/vol alcohol (EtOH) for a period of 2 hours. The dosing regimen, developmental stage and length of exposure, and concentrations of alcohol delivered precisely followed those of prior studies that have found significant behavioural changes but no gross physical abnormalities (Fernandes & Gerlai, 2009; Buske & Gerlai, 2011; Mahabir et al, 2014). Eggs were maintained in the incubator during the alcohol exposure period. Following alcohol exposure, the eggs were rinsed with system water three times to remove any residual alcohol, and were placed back into glass containers containing system water in the incubator until 5 days post-fertilization. Larval fish were subsequently placed into rearing tanks of a system rack, and housed in groups of 10–15 fish per 3 L tank, each tank containing fish of the same concentration group. As fish grew larger, they were separated into smaller groups of 5 per 3L tank until they reached adulthood and behavioural testing began.

Housing

The system rack that contained the rearing/holding tanks was a high density (10 fish per 3 litre tank) recirculating water system (Aquaneering Inc.) which utilizes biological, mechanical and activated carbon filters as well as a UV sterilizing unit. The water temperature was maintained at 27°C by internal heaters controlled by thermostat. System water of the recirculating high density rack was reverse osmosis filtered and reconstituted to the appropriate salinity (300 micro-Siemens conductivity) by adding 60 mg/l of sea salt (Instant Ocean, Aquarium Systems Inc., Mentor, OH, USA). The same room was used for both housing and experimental testing of the fish. The room was illuminated by fluorescent light tubes from the ceiling. Fish were kept on a 12h light:dark cycle with lights turned on at 0900 hour. Fry were initially fed Larval Artificial Plankton 100 (Particle size below 100 µm, ZeiglerBros Inc., Gardners, PA, USA) and subsequently freshly hatched brine shrimp nauplii (*Artmeia salina*). Starting at 3 weeks of age juvenile fish were fed commercial flake food

twice a day (Tetramin Tropical fish flake food, Tetra Co, Melle, Germany) supplemented with live brine shrimp *napulii*.

Behavioural Apparatus

Fish were individually tested in either a long tank (100 cm × 25cm × 30cm, length × width × height) or short tank (50cm × 25cm × 30 cm, length × width × height). The bottom and back sides of each tank were lined with green Plexiglas in order to create a more naturalistic environment for the fish, and to reduce anxiety not elicited by the novel tank or predator stimulus. The remaining sides of the tank were transparent, and each flanked by a Dell computer monitor. Each computer monitor was connected to a Dell Vostro 1520 laptop. This set-up was used to display a blank black screen (for the duration of the habituation period) or the sympatric predator stimulus (computer animated image of a clown knifefish, *Chitala ornate*, presented multiple times intermittently after the habituation period) using an in-house developed custom software application (Qin et al., 2014). This test apparatus was illuminated by an overhead 13W fluorescent aquarium light tube. All trials were recorded using a SONY HDR-XR550 Handycam camcorder positioned on a tripod facing the long side of the tank. All recorded trials were subsequently replayed and analyzed using Noldus Ethovision Version 8 (Noldus Information Technology, Wageningen, The Netherlands), which allowed us to track the swim path of experimental fish and extract numerous path parameters.

Behavioural testing procedure and experimental design

Fifty percent of the experimental fish were randomly assigned to the long tank (100 cm long) and the other fifty percent to the short tank (50cm long). Behavioural responses were measured during a 21-minute testing session. Each experimental zebrafish was measured only once. Fish were netted singly into a novel tank and their behaviour was monitored for 10 min, the habituation period. Subsequently, fish were presented with an animated (moving) computer image of a predator. During the habituation period both computer monitors displayed a blank black screen for 10 minutes. This period served two purposes. One, as a result of handling stress and of being placed in a novel tank, zebrafish are expected to exhibit anxiety-like responses (Stewart et al., 2012). Two, by the end of the 10 min habituation period, the experimental fish should have reached a stable baseline (habituated state) to which subsequent predator stimulus induced responses may be compared. Following the novel habituation period, a computer animated image of a sympatric zebrafish predator, the clown knifefish, was displayed on one computer screen for 1 minute while the other monitor remained blank. The knifefish is a slowly moving ambush predator and the animation was set to mimic the natural speed of this fish (about 0.5 – 1 cm/sec). This first predator presentation was followed by a 3-minute inter-trial interval (ITI) during which both screens remained black. A second predator stimulus was shown for 1 minute, and this was again followed by a 3-minute ITI, which was followed by a third and final 1-minute predator presentation and subsequent two minutes of blank screens. The predator stimulus was only presented on one screen at a time. Presentation occurred either all on the left screen, all on the right screen or on alternating screens (left, right, left or right, left, right), and this was counterbalanced across experimental fish to control for any possible side bias. Subjects were tested once and only in one condition. After the completion of the behavioural recording

session tested fish were placed in a holding tank separate from untested subjects. Using alternating presentations of the predator allowed observation of whether fish were actively responding to the predator each time, an active avoidance reaction, as opposed to remaining on the first “safe” side of the tank, a passive avoidance reaction.

We quantified the following swim path parameters separately for the novel tank habituation period and the predator presentation period. Total distance swam is the distance the fish swam during these respective periods. In case of active avoidance due to elevated fear or anxiety the value of total distance may increase, whereas in case of passive avoidance, it is expected to decrease. Angular velocity is a measure of how fast and how sharp the fish turned, a response that has been found to correlate well with the behavioural element, erratic movement in zebrafish (Bass & Gerlai, 2008; Gerlai et al., 2009; Gerlai, 2010). The value of angular velocity is expected to increase in response to aversive stimuli or in aversive contexts. Variance of distance to the bottom of the tank is a measure of vertical exploration, with a high variance value signifying more changes in the vertical position of the fish in the water, and lower variance values corresponding to more consistent vertical positioning of the fish (Tran & Gerlai, 2013). Vertical exploration is expected to decrease under aversive conditions in zebrafish (Gerlai, 2010).

Statistical analysis

SPSS version 17 was used to perform statistical analyses. First, an overall repeated measures variance analysis (ANOVA) was performed with Interval as the repeated measure within subject factor (2 levels: novel tank and predator exposure intervals), Tank Size (a between subject factor with 2 levels: long and short tank), and alcohol Concentration (a between subject factor with 5 levels: 0.0, 0.25, 0.50, 0.75 and 1.00 vol/vol % embryonic bath alcohol concentration). In case of significant main or interaction effects, subsequent two and/or uni-factorial follow up ANOVAs were conducted as well as *post-hoc* Tukey HSD tests were performed. Effects of tank size and alcohol concentration were compared using a multivariate ANOVA to test for main and interaction effects with follow up ANOVAs and post-hoc Tukey HSD tests performed when appropriate. We accepted significance when the probability of null hypothesis was less than 5% ($p < 0.05$).

RESULTS

Three factorial repeated measures ANOVA of the total distance swum (figure 1) found Interval to have a significant effect ($F(1,138) = 66.307$, $p < 0.001$). However, the interaction terms Interval $\times$ Tank Size and Interval $\times$ Concentration were found non-significant. The triple interaction term Interval $\times$ Tank Size $\times$ Concentration was found to border significance ($F(4, 138) = 2.201$, $p = 0.072$). Tank Size was found to have a significant effect ($F(1,138) = 12.811$, $p < 0.001$), but the effect of Concentration and the interaction term Tank Size $\times$ Concentration were found non-significant. Because the triple interaction term was bordering significance, and because ANOVA has been shown to be insensitive (underpowered) to detect the significance of interaction between main factors (Wahlsten, 1990), we decided to conduct follow up statistical analyses. First, we performed an ANOVA separately for results obtained in the long and the short tank. In the long tank, we confirmed

Interval to have a significant effect ($F(1,69)=18.956$, $p<0.001$), but also importantly found the Interval $\times$ Concentration interaction highly significant ($F(4, 69)=3.566$, $p=0.011$). We also confirmed the effect of Concentration to be non-significant. In the short tank, the effect of Interval was significant ($F(1, 69)=62.711$, $p<0.001$), but the effect of Concentration and the Interval $\times$ Concentration interaction term were both non-significant. To further investigate the significant Interval $\times$ Concentration interaction found in the long tank, we conducted a unifactorial ANOVA for each interval (initial exposure period to novel tank alone versus period during which the predator image was shown) separately. This analysis revealed no significant differences among the alcohol concentration groups for the novel tank. When examining total distance swam during this period alone, a significant effect of Tank Size was found ($F(1,139)=5.391$, $p=0.022$) with fish swimming more in the long tank than in the shorter tank. However, no significant effects of Concentration, nor Concentration $\times$ Tank Size effects were found. During the predator stimulus presentation interval, a significant alcohol effect was found ($F(4, 70)= 3.400$, $p=0.012$). Subsequent Tukey HSD post-hoc test revealed a significant difference ($p=0.010$) between distance swam by fish in the 0.50% EtOH group compared to fish in the 0.75% EtOH group, with fish in 0.50% group swimming an average of $128.9 \text{ cm} \pm 38.3 \text{ cm}$ more than fish in the 0.75% EtOH group. Other group differences were found non-significant ($p > 0.05$).

Three factorial repeated measure variance analysis of angular velocity (figure 2) found the effect of Interval significant ($F(1,140)= 36.754$, $p<0.001$), but detected no significant interaction between this factor and any other main factors. The effects of Tank Size, Concentration and the Tank Size $\times$ Concentration interaction terms were non-significant. During the novel tank period alone, no significant main or interaction effects were found. Considering these statistical findings and the results presented on Figure 3, we conclude that presentation of the predator stimulus consistently increased angular velocity, an effect that was independent of prior embryonic alcohol treatment and also of the length of the tank in which the fish were tested.

Three factorial repeated measure ANOVA for Variance in distance to bottom (figure 3) found the effect of Interval significant ($F(1,138)=3.644$, $p<0.001$), but no significant interactions (Interval $\times$ Tank Size, Interval $\times$ Concentration, or Interval $\times$ Tank Size $\times$ Concentration) were found. No significant between-subject main effects of Tank Size or Concentration were found. When examining the novel tank period, no significant main or interaction effects were found. With these findings, we conclude that the presentation of the predator image led to a consistent decrease in variance in distance to the bottom independently of tank size or dose of embryonic alcohol exposure.

Figure 4 shows the distance to the stimulus screen that presented the animated predator image during the stimulus presentation period. Three factorial repeated measures variance analysis of distance to stimulus screen found a significant Interval effect ($F(1,139)= 47.933$, $p<0.001$), but the Interval $\times$ Tank Size, Interval $\times$ Concentration and Interval $\times$ Tank Size $\times$ Concentration interaction terms were non-significant. The effect of between subject factor, Tank Size, was found significant ($F(1,139)=73.022$, $p<0.001$), but the effect of Concentration and the interaction term Tank Size $\times$ Concentration were both found non-significant. Based on these results, we conclude that the presentation of the predator

stimulus led to a consistent increase of distance to stimulus side independently of the dose of prior embryonic alcohol exposure, and that this predator effect was observable in both the long and the short tank.

DISCUSSION

The purpose of this study was to determine whether responses to aversive contexts or stimuli are altered in adult zebrafish by previous exposure of these fish to low concentrations of alcohol for a short period of time during their embryonic development. That is, we were interested in investigating whether the impaired reactions towards social stimuli in embryonic alcohol exposed zebrafish observed previously (Fernandes & Gerlai, 2009; Buske & Gerlai, 2011) are due to altered fear/anxiety. Behavioural responses to diffuse aversive stimuli are often regarded as anxiety or anxiety-like responses, whereas responses to an aversive stimulus whose on-set and off-set is clearly determined are considered fear or fear-like responses (Gerlai, 2010; Maximino et al., 2010; Stewart et al., 2012). In zebrafish research, anxiety is thus often measured as responses elicited by exposure to a novel environment (Stewart et al., 2012; Bencan et al, 2009; Sackerman et al 2010), whereas fear is quantified as responses, for example, to the appearance of predators or their moving images (Gerlai et al., 2009; Gerlai, 2010; Ahmed et al., 2011; Ahmed et al., 2012). In the current study, we found no alterations in the responses to either of these aversive situations among the embryonic alcohol exposed fish compared to their control counterparts. For example, no difference between control and embryonic alcohol exposed fish was found in any behaviours during the habituation period. The predator stimulus induced behavioural responses were also statistically indistinguishable between control and alcohol exposed fish except for one behaviour: overall swimming activity was found elevated in response to the predator image in 0.50% alcohol treated fish in the long tank. The interpretation of this finding, however, requires further research because other behavioural responses associated with fear (including the distance to stimulus and angular velocity) did not show the alcohol effect, and we also did not find elevated activity in these fish in the short tank. In summary, our results are compatible with the conclusion that embryonic alcohol treatment did not alter behavioural responses to aversive contexts (novelty) and stimuli (predator image) in adult zebrafish. Thus, we conclude that the previously reported robust social response deficit found in embryonic alcohol exposed fish is unlikely to be due to altered fear or anxiety of these fish. Does the lack of alteration in fear and anxiety related behaviours in the zebrafish FASD model match what has been found in human FASD patients?

The relationship between fetal or neonatal alcohol exposure and later life anxiety has been controversial or complex at best in mammals, including humans suffering from FASD (Barr et al 2006) and rodent models of FASD (Hellemans et al, 2010b). The complexity is due to many factors. For example, the effect of alcohol exposure appears to be highly dose dependent with lower concentrations often not having any effect on anxiety responses in later life in humans (Jacobson et al, 1999) or in zebrafish (Baiamonte et al., 2016 versus this study). The association between embryonic alcohol exposure and changes in anxiety later in life is also dependent upon the developmental stage at which the embryo was exposed to this substance (Zhang et al 2005). Last, the effect of embryonic alcohol exposure on anxiety related responses has also been found to depend upon stressors the subject was exposed to

later in life (Hellemans et al, 2010). Could a relatively simple behavioural test as the one employed in this study, conducted with a simple vertebrate as zebrafish, allow one to tap into such complexity?

The answer to this question is likely no. Nevertheless, previously, zebrafish have been shown to possess a rich antipredatory/fear/anxiety behavioural repertoire that was found to be aversive stimulus and context dependent (Gerlai, 2010; Luca & Gerlai, 2012). Furthermore, our results confirm that robust behavioural responses may be induced in zebrafish both by exposing them to novelty, and by presenting them with an animated image of a sympatric predator. The behavioural responses we quantified represent typical aversive or antipredatory reactions. These responses include both active avoidance (increased distance from the predator and increased angular velocity) and passive avoidance (reduction of activity and reduced variance in distance to bottom). Increased angular velocity has been found to correlate strongly with erratic movement, a motor pattern characterized by a series of fast swim episodes with quick swim direction changes, believed to confuse aerial and aquatic predators and/or to lead to stirring up debris on the bottom, which would hide the prey (Bass & Gerlai, 2008; Gerlai, et al., 2009). Reduced activity may also be an effective avoidance response as it minimizes the probability of being detected by the predator. Do these avoidance responses differ under different aversive contexts? We investigated this question using two differently sized experimental tanks, assuming that the longer tank would bias the responses towards more active and the shorter tank towards more passive avoidance reactions.

Our findings partially confirmed our expectations. Tank size significantly influenced behavioural responses of the experimental zebrafish. For example, in the longer tank, zebrafish swam more, and remained further away from the predator stimulus compared to how they behaved in the shorter tank. However, notably, the effect of the animated image of the sympatric predator was independent of, i.e. did not interact with, tank size. We also found no interaction between tank size and the effect of embryonic alcohol treatment. Thus, finding no embryonic alcohol effects in both experimental tanks and also both during the habituation and predator periods strongly suggest that exposure of zebrafish to low concentrations of alcohol for only 2 hours at 24th hour post-fertilization does not lead to long-lasting changes in fear or anxiety-like responses in the exposed fish. This negative finding thus suggests that similarly to motor or perceptual function (Fernandes & Gerlai, 2009; Buske & Gerlai, 2011), fear and anxiety may also be excluded from factors underlying the impaired response to social stimuli found previously in embryonic alcohol exposed fish. What may be behind this alteration then?

It is possible that the exposed zebrafish are unable to recognize other zebrafish (Buske & Gerlai, 2011) or the images of zebrafish (Fernandes & Gerlai, 2009) as their conspecifics. It is also possible that although the affected zebrafish recognize other zebrafish as their potential shoal-mates, they are not motivated to shoal with them. The latter possibility is supported by finding significant impairment in the dopaminergic system of embryonic alcohol exposed zebrafish (Buske & Gerlai, 2011; Mahabir et al., 2014; Fernandes et al., 2015). Buske & Gerlai (2011) found that the embryonic alcohol exposed zebrafish had smaller amount of dopamine and DOPAC in their brains when tested at their adult stage.

Mahabir et al. (2014) discovered that embryonic alcohol treated zebrafish suffer from a retarded development of the dopaminergic system as these fish did not increase the relative levels of dopamine and DOPAC during development as control fish did. Last, Fernandes et al. (2015b) showed that control zebrafish respond to animated images of their conspecifics with a robust increase of dopamine and DOPAC levels in their brain, a response that was completely abolished in adult zebrafish that were exposed to 1% alcohol for 2 hours at their 24th hour post fertilization stage. Dopamine is known to mediate reward in mammals (Gunaydin & Deisseroth, 2014) and in zebrafish (Saif et al., 2013; Scerbina et al., 2012), and thus the correlation found between the impaired dopaminergic responses and the impaired shoaling response seen in embryonic alcohol exposed fish may represent a causal relationship. It is also notable, that embryonic alcohol exposed zebrafish were found to show no impairment in their motivation to work for food in a learning task (Fernandes, et al, 2014), suggesting that the impaired motivation seen in the shoaling tasks may be specific to these tasks. The latter raises the intriguing possibility that the impaired shoaling response seen in adult zebrafish treated with alcohol during embryonic development is the result of impaired social behaviour, a deficit that would parallel that seen in human FASD (Stevens et al, 2012).

Although the above working hypotheses require future systematic empirical studies, our current results suggest that fear and anxiety are unlikely explanations for the impaired social behavioural responses observed in zebrafish exposed to alcohol during their embryonic development. These results also demonstrate that zebrafish may be a useful tool with which the effects of embryonic alcohol exposure may be further investigated.

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Figure 1.

Total distance experimental zebrafish swam was generally reduced in response to the presentation of the animated image of a sympatric predator, the clown knifefish (Panel A long tank, panel B short tank) except in the 0.50% embryonic alcohol treated fish measured in the long tank. Mean $\pm$ SEM are shown. Black bars show performance during the novel tank test period when the stimulus screen was blank. Grey bars show performance during stimulus presentation when the stimulus screen was showing the animated image of the predator.

Figure 2.

Angular velocity was increased in response to the presentation of the animated image of a sympatric predator, the clown knifefish both in the long (Panel A) and in the short (panel B) tank. However, this increase was independent of embryonic alcohol treatment. Mean $\pm$ SEM are shown. Black bars show performance during the novel tank test period when the stimulus screen was blank. Grey bars show performance during stimulus presentation when the stimulus screen was showing the animated image of the predator.

Figure 3.

Variance in distance to bottom was increased in response to the presentation of the animated image of a sympatric predator, the clown knifefish both in the long (Panel A) and in the short (panel B) tank. However, this increase was independent of embryonic alcohol treatment. Mean $\pm$ SEM are shown. Black bars show performance during the novel tank test period when the stimulus screen was blank. Grey bars show performance during stimulus presentation when the stimulus screen was showing the animated image of the predator.

Figure 4.

Distance of experimental zebrafish to the stimulus screen was reduced in response to the presentation of the animated image of a sympatric predator, the clown knifefish both in the long (Panel A) and in the short (panel B) tank. However, this reduction of distance was independent of embryonic alcohol treatment. Mean $\pm$ SEM are shown. Black bars show performance during the novel tank test period when the stimulus screen was blank. Grey bars show performance during stimulus presentation when the stimulus screen was showing the animated image of the predator.