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Impairment of social behaviour persists two years after embryonic alcohol exposure in zebrafish: A model of fetal alcohol spectrum disorders

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HIGHLIGHTS

- Zebrafish embryos were exposed to 1% bath ethanol at 24 hpf for 2 h.
- Shoaling response to animated conspecific images was quantified.
- Embryonic exposure impaired shoaling response in 2 year old fish.
- Exposure of embryos to even low dose of ethanol has long lasting negative effects.

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ABSTRACT

Zebrafish naturally form social groups called shoals. Previously, we have shown that submerging zebrafish eggs into low concentrations of alcohol (0.00, 0.25, 0.50, 0.75 and 1.00 vol/vol% external bath concentration) during development (24 h post-fertilization) for two hours resulted in impaired shoaling response in seven month old young adult zebrafish. Here we investigate whether this embryonic alcohol exposure induced behavioural deficit persists to older age. Zebrafish embryos were exposed either to fresh system water (control) or to 1% alcohol for two hours, 24 h after fertilization, and were raised in a high-density tank system. Social behaviour was tested by presenting the experimental fish with a computer animated group of zebrafish images, while automated tracking software measured their behaviour. Control fish were found to respond strongly to animated conspecific images by reducing their distanceand remaining close to the images during image presentation, embryonic alcohol treated fish did not. Our results suggest that the impaired shoaling response of the alcohol exposed fish was not due to altered motor function or visual perception, but likely to a central nervous system alteration affecting social behaviour itself. We found the effects of embryonic alcohol exposure on social behaviour not to diminish with age, a result that demonstrates the deleterious and potentially life-long consequences of exposure to even small amount of alcohol during embryonic development in vertebrates.

1. Introduction

Exposure of the developing fetus to alcohol (ethyl alcohol or ethanol) produces disorders collectively labeled under the umbrella term fetal alcohol spectrum disorder (FASD) [1–3]. Within FASD, fetal alcohol syndrome (FAS) is the most severe category, and is associated with heavy alcohol consumption and/or lengthy exposure during gestation. Furthermore, FAS is characterized by facial abnormalities, growth deficiencies and central nervous system deficits [3–5]. Alcohol related neurodevelopmental disorder (ARND) is believed to result from exposure to lower doses of alcohol compared to FAS, and is considered to be a milder disorder within FASD. Patients with ARND exhibit behavioural and cognitive abnormalities that are similar to the problems seen in individuals with FAS, but they lack the severe physical aberrations required for the FAS diagnosis [2,3]. Cognitive deficits, impaired academic performance, abnormal emotional functioning, and maladaptive social behaviour are common outcomes across the spectrum of

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FASD patients [6–8]. Approximately 1 in 100 children born in the Western world suffer from FASD, and the largest proportion of these children fall under the category of ARND [9,10]. Thus understanding the mechanisms underlying FASD, and particularly ARND, is of crucial importance.

The zebrafish is a powerful animal model [11] well suited for FASD research. A female zebrafish can produce 200–300 eggs every other day [12]. Because these eggs are small, a large number of them can be accurately exposed to alcohol at precise developmental stages and for specific durations, thereby reducing potential error variation among the exposed subjects [13]. Egg fertilization and development occur outside of the mother, and the fry grow without parental care [14]. Thus potential confounds associated with the intra-uterine environment or with maternal care, characteristic of mammalian species, are absent in the zebrafish [15]. The zebrafish chorion is partially permeable to alcohol [16,17], which makes the alcohol exposure procedure extremely simple: eggs may be submerged in the desired alcohol concentration for the chosen length of time at the desired stage of development [1].

The powerful recombinant DNA-based techniques available for the zebrafish together with its biological features that are often very similar to those of mammals make this species a suitable animal model for FASD research. For example, several forward and reverse genetic tools have been developed for zebrafish [18] allowing efficient discovery and functional analysis of novel genes [14]. The sequencing of the entire zebrafish genome has been achieved [19]. High nucleotide sequence homology and functional similarities between the zebrafish and mammalian genes have also been demonstrated [19,see review by 20]. Last, zebrafish have a sophisticated vertebrate brain, sharing basic anatomical layout [21] and neurochemistry features [22] with those of higher order vertebrates including mammals. All these features of the zebrafish make this species an excellent candidate for the mechanistic exploration of human conditions, including FASD [1,13,23,24].

A devastating outcome of embryonic alcohol exposure is the life-long suffering caused by cognitive and behavioural deficits [Kodituwakku:2007dk; 25]. Clinical studies have shown that children with FASD have problems with social skills [7,8,26,27] and that these deficits continue into adolescence [28] and adulthood [25]. Recently, Hamilton et al. found impaired social behaviour in Long-Evans rats exposed to alcohol prenatally to persist into adulthood [29].

Zebrafish have a genetic predisposition to form groups, a behaviour called shoaling [30]. Furthermore, when a single zebrafish is presented with a live group of zebrafish or a computer generated animated group of images of zebrafish [4], the lone fish rapidly decreases the distance between itself and the group, and subsequently maintains this short distance as long as the shoaling stimulus is present, a behaviour we call the "shoaling response." We use the shoaling response as a measure of social behaviour, where a strong response (large decrease between the single experimental fish and the group) is indicative of an appropriate social behaviour, and a weak response (small or no decrease in distance) is indicative of impaired social behaviour. In the past we have shown that embryonic alcohol exposure impaired social behaviour in adult zebrafish [1,31]. Specifically, we found a dose-dependent decrease in the shoaling response, i.e., control fish demonstrated a robust reduction in the distance towards the stimulus, while alcohol treated fish did not [1,31]. Notably, these fish were exposed to a mild alcohol dose only once during development (24 post fertilization) for a short duration (2 h), while their social behaviour was tested at the age of seven month [1] or eight months [31].

The goal of the current study was to determine whether the social behaviour impairment seen in young adult zebrafish reported previously [1,23,31] remained observable in older fish, i.e. in twenty four-month-old experimental subjects. Zebrafish exhibit signs of age dependent changes similar to mammals [32], but it is not known whether the effect of embryonic alcohol exposure continues to manifest beyond the young adult stage (i.e. beyond seven or eight months). In the current study, zebrafish were exposed to 1.0% alcohol for two hours at the 24th h post-fertilization stage. Using an automated experimental paradigm we induced and quantified social behaviour by measuring the distance of the experimental fish from a computer controlled animated group of five zebrafish (moving conspecific images, the animated shoal). Here, we report that twenty four-month-old zebrafish exposed to alcohol during their embryonic development exhibit a significantly diminished response to the animated shoal, while control (freshwater exposed) fish show a robust response to the animated shoal. We also report that the impairment observed in the alcohol treated fish is not accompanied by deficits in motor function nor it is likely caused by altered visual perception.

2. Methods

2.1. Animals and housing

Sexually mature adult zebrafish of the AB strain were bred at the University of Toronto, Mississauga Vivarium (Mississauga, Ontario, Canada) to obtain fertilized eggs. The progenitors of this population were obtained from the ZFIN Center (Eugene, Oregon, USA). AB is one of the most frequently studied zebrafish strains, which is often used in forward genetic (mutagenesis) studies [33]. Approximately 80 fertilized eggs were collected two hours post-fertilization (hpf) and washed with system water: deionized and sterile water supplemented with 60 mg/L Instant Ocean Sea Salt (Big Al's Pet Store, Mississauga, ON, Canada).

The low alcohol concentration and short exposure time were chosen to model the low levels and frequency of drinking more commonly seen during pregnancy that are associated with ARND. An equal number of eggs (approximately 40) were placed in containers with 100 mL of solution of the corresponding alcohol concentration (0 or 1.00 vol/vol%) for two hours. The eggs were subsequently washed with system water and maintained in 1.3 L tanks that were part of a nursery rack (Aquaneering Inc., San Diego, CA). Upon hatching, the fry were fed Larval AP 100 food (Zeigler-Bros. Inc., Gardners, PA). After three weeks, zebrafish were moved to 2.8 L rearing tanks (Aquaneering Inc.) placed in a high-density rack system. The system had multistage filtration that contained a mechanical filter, a fluidized glass bed biological filter, an activated carbon filter, and a fluorescent UV light-sterilizing unit. Ten percent of the system water was replaced daily. While the fish were in the tanks of the high-density racks, they received a mixture of dried fish food (4 parts of Nelson Silver Cup, Aquaneering Inc. and powered spirulina, 1 part, Jehmco Inc., Lambertville, New Jersey). Fluorescent light tubes mounted on the ceiling provided illumination. Lights turned on at 08:00 h and off at 21:00 h. Zebrafish were housed in the tanks of the system rack in groups of 10–12 until they were 24 months old. The sample sizes of treatment groups were as follows: 0.00% EtOH control, $n = 26$ and 1.00% EtOH $n = 17$. Over the course of two years we experienced attrition in sample size due to naturally occurring death within groups.

2.2. Behavioural apparatus

The experimental set up consisted of a 37 L tank ($50 \times 25 \times 30$ cm, length × width × height) with a flat LCD computer screen (17 inch Samsung SyncMaster 732N monitor) placed on the left and right side of the tank along the width of the tank. Each monitor was connected to a Dell Vostro 1000 Laptop Running a custom made software application [2][see 2] that allowed the