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Ethanol effects on three strains of zebrafish: model system for genetic investigations

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Abstract

The effects of acute and chronic ethanol administration on the wild-type (WT), long-fin striped (LFS), and blue long-fin (BLF) strains of zebrafish were investigated. In the LFS strain, acute exposure to 0.25% (v/v) ethanol inhibited the startle reaction and increased both the area occupied by a group of subjects and the average distance between each fish and its nearest neighbor. Similar effects were found in the WT fish although higher concentrations of ethanol were required. No effects on the behavior of the BLF fish were observed with up to 1.0% (v/v) ethanol. Brain alcohol levels were comparable among the three strains precluding a pharmacokinetic explanation for the behavioral results. In LFS zebrafish, behavioral tolerance was observed after 1 week of continual exposure to ethanol. Conversely, chronic ethanol exposure of the WT fish for up to 2 weeks did not result in the development of tolerance, but rather appeared to increase the disruptive action of the drug. The present results suggest the observed strain differences in the effects of ethanol reflect genotypic differences in both the response of the central nervous system (CNS) to ethanol as well as the ability of the CNS to adapt to ethanol exposure. Although preliminary, the present study indicates that the zebrafish is an excellent model system to investigate the genetic determinants involved in regulating the responses to ethanol.

Keywords: Ethanol; Zebrafish; Genetics; Tolerance

1. Introduction

Ethanol exerts a variety of actions on the central nervous system (CNS). Behaviorally, the presence of ethanol impairs motor coordination, sensory perception and cognition as well as being anxiolytic and inducing sedation and hypothermia (Charness et al., 1989; Fleming et al., 2001). With chronic ethanol exposure, neuronal adaptation leads to physical dependence and tolerance as well as neurotoxicity (Charness et al., 1989; Fleming et al., 2001). The development of ethanol-related tolerance, a diminished response with repeated exposure, may increase consumption and play a role in the etiology of alcoholism (Tabakoff and Hoffman, 1988).

The effects of ethanol are influenced by the genotype of the subject. Genetic factors play an important role in the potential for an individual to develop alcoholism (Vanyukov and Tarter, 2000). Similarly, strain differences and selective breeding demonstrate the importance of genetic determinants in regulating the effects of ethanol in rodent models. For example, strain differences in rodents are found with respect to ethanol consumption as well as with ethanol-induced hypothermia and ataxia (Crabbe et al., 1982; Belknap et al., 1993; Phillips and Crabbe, 1991). Furthermore, selected breeding has resulted in the development of rodent lines with phenotypic differences in severity of withdrawal, ethanol consumption, as well as in ethanol-induced sedation, hypothermia, and locomotor activity (Phillips and Crabbe, 1991; Crabbe et al., 1994).

A variety of approaches have been used to identify the genes and gene products responsible for influencing the effects of ethanol. These methods include analysis of selectively bred rodent lines, quantitative trait loci mapping of recombinant inbred strains, development of transgenic ani-

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mals, and, more recently, the use of microarrays. Although these methods have significantly enhanced our understanding of the determinants of ethanol sensitivity and effects, they have the disadvantages of being costly and time consuming. Furthermore, while candidate genes potentially involved with regulating the response to ethanol have been identified, the number of unidentified ethanol-sensitive genes is still large and further work is needed to clarify the role of these genes.

The zebrafish, *Danio rerio*, is a small freshwater teleost that has been extensively used for biomedical research, especially developmental studies. Although zebrafish offer a number of advantages for genetic studies, very few studies has used the zebrafish model to study the effects of ethanol (Laale, 1971; Baumann and Sander, 1984; Blader and Strähle, 1998; Gerlai et al., 2000). The present study was undertaken to assess the suitability of using zebrafish as a model system to investigate the acute and chronic effects of ethanol. In addition, the influence of genotype was studied.

2. Methods

2.1. Animal model and treatment

Approximately 450 wild-type (WT), long-fin striped (LFS), and blue long-fin (BLF) zebrafish, *D. rerio*, were used in this study. WT zebrafish are 3 cm in length and are characterized by steel-blue body stripes. LFS zebrafish are 3.25 cm in length and are identical in appearance to WT zebrafish except for long dorsal and pectoral fins. BLF zebrafish are 3.8 cm in length with a single, less pronounced stripe, a bluish-grey color, and long pectoral and dorsal fins. Young adult zebrafish were obtained from The Fish Place (North Townawanda, NY), Markheim Tropical Fish and Pet Store (Amherst, NY), and Blackwinds Pet Supply (Niagara Falls, NY) at least 2 weeks prior to testing. Fish were not separated by gender, but each strain was housed separately in a 10-gal tank containing dechlorinated, filtered, tap water heated to 74 °F (24 °C). Fish diet consisted of flake fish food supplemented with live brine shrimp. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University at Buffalo. Neither chronic nor acute ethanol treatment resulted in fish mortality.

For acute ethanol studies, groups of WT, LFS, and BLF zebrafish were removed from the home tank and transferred into a 10-l temperature-regulated tank filled with either 0.25%, 0.5%, or 1.0% (v/v) ethanol. The concentrations of ethanol were based on pilot study. For behavioral studies, fish were tested after a 2-h exposure to ethanol. Controls for the acute ethanol experiments consisted of other fish removed from the home tank and tested behaviorally.

For chronic ethanol studies, WT and LFS zebrafish were transferred to a 5-gal covered aquarium containing 0.5% (v/v) ethanol. The fish remained within the aquarium for up to 2 weeks and were removed only for behavioral testing. The concentration of ethanol in the tank was monitored daily and adjusted as needed. Baseline behavior was assessed prior to the chronic ethanol treatment by measuring the distance between each fish and its nearest neighbor for a 30-min period prior to treatment.

2.2. Behavioral apparatus

The behavioral apparatus consisted of a bowl 20 cm in diameter containing 700 ml of the appropriate concentration of ethanol or aquarium water. A 324-cm^2 grid subdivided into 36 blocks (9 cm^2/block) was centered under the bowl. A digital imaging system consisting of an Intel digital camera, a computer, and the Intel Create and Share (Intel, Hudson, MA, http://www.intel.com/) software program were used to record the swimming behavior and startle reaction of the fish.

2.3. Startle reaction

Prior to testing, individual fish were placed in the above behavioral apparatus and allowed to acclimate to their surroundings. The startle reaction was assessed by dropping a 1-cm diameter glass bead attached to a string in front of each fish. When the glass bead was dropped, each fish was in the middle of the bowl approximately 13 cm from its edge with its head facing the edge of the bowl. As the bead was dropped and for several seconds thereafter, the movements of each fish were videotaped with an Intel digital camera. Data were analyzed by quantifying the number of 9 cm^2 blocks on the grid that each fish traversed as it moved away from the glass bead. The startle response was recorded only once/fish during the acute studies.

2.4. Swimming behavior

Groups of zebrafish were placed in the above behavioral apparatus for 30 min during which time a digital imaging system recorded the fish at 30-s intervals. For acute ethanol treatment, two test groups ($n = 8$/group) of WT and LFS zebrafish but only one group ($n = 7$/group) of BLF fish were used. For chronic ethanol treatment, the same group of eight WT and eight LFS zebrafish were tested prior to the ethanol treatment and following 1 and 2 weeks of ethanol exposure.

Stored images were analyzed with the Image Tool program (http://ddsdx.uthscsa.edu/dig/itdesc.html) to determine the distance between each fish and its nearest neighbor, and the mean area that each group of fish occupied. The nearest neighbor distance was obtained by connecting the most cranial midpoint of each fish with the most cranial midpoint of its closest neighbor. The distance between each fish and its nearest neighbor was determined on every third image within the series of 60 captured images. A mean was determined for each frame and test group. Test groups were averaged to determine a mean value for each concentration

of ethanol within the acute study. The mean area occupied by the group of fish was determined from a polygon created by connecting the most cranial midpoints of the peripheral fish within each test group. Individual values were averaged to determine the mean area for each group.

2.5. Brain alcohol levels

Zebrafish were anesthetized with ice and exsanguinated. The brains were removed, weighed, and then homogenized in 100 μl of 3.5% perchloric acid. The homogenate was centrifuged at 15,000 × *g* for 5 min, and an aliquot of the supernatant was used to determine brain alcohol content. Brain alcohol levels as well as the concentration of alcohol within the tanks were determined enzymatically using alcohol dehydrogenase and measuring the production of NADH spectrofluorometrically (Rabin et al., 1987).

2.6. Statistics

Means (±S.E.M.) were determined for the startle reaction, nearest neighbor distance, and mean area occupied by averaging values in each test group or groups. Data from acute studies were tested with Student's two-tailed *t* test and ANOVA. Data from chronic studies were tested with ANOVA followed by Tukey's post hoc test. An alpha level of .05 was used to determine statistical significance.

3. Results

3.1. Acute ethanol exposure

All WT, LFS, and BLF control zebrafish demonstrated a swimming pattern in which fish appeared clustered (Fig. 1), moving in unison and in close proximity with one another. Ethanol-treated WT and LFS strains, however, appeared less clustered with a larger distance between each fish and its nearest neighbor and a greater area of the test tank being occupied by each ethanol-treated group (Fig. 1). These observations were supported by an ethanol concentration-dependent increase in nearest neighbor distance and in the area occupied by each WT group. Increases in the nearest neighbor distance (Fig. 2A) reached significant levels with 0.5% (v/v) ethanol ($t = -4.654$, $df = 120$, $P < .001$). A significant increase in the area occupied by the WT strain was detected with 0.25% (v/v) ethanol ($t = -4.199$, $df = 273$, $P < .001$) (Fig. 3A). LFS behavior appeared similarly affected by acute ethanol treatment. A concentration-dependent increase in nearest neighbor distance in LFS fish was first

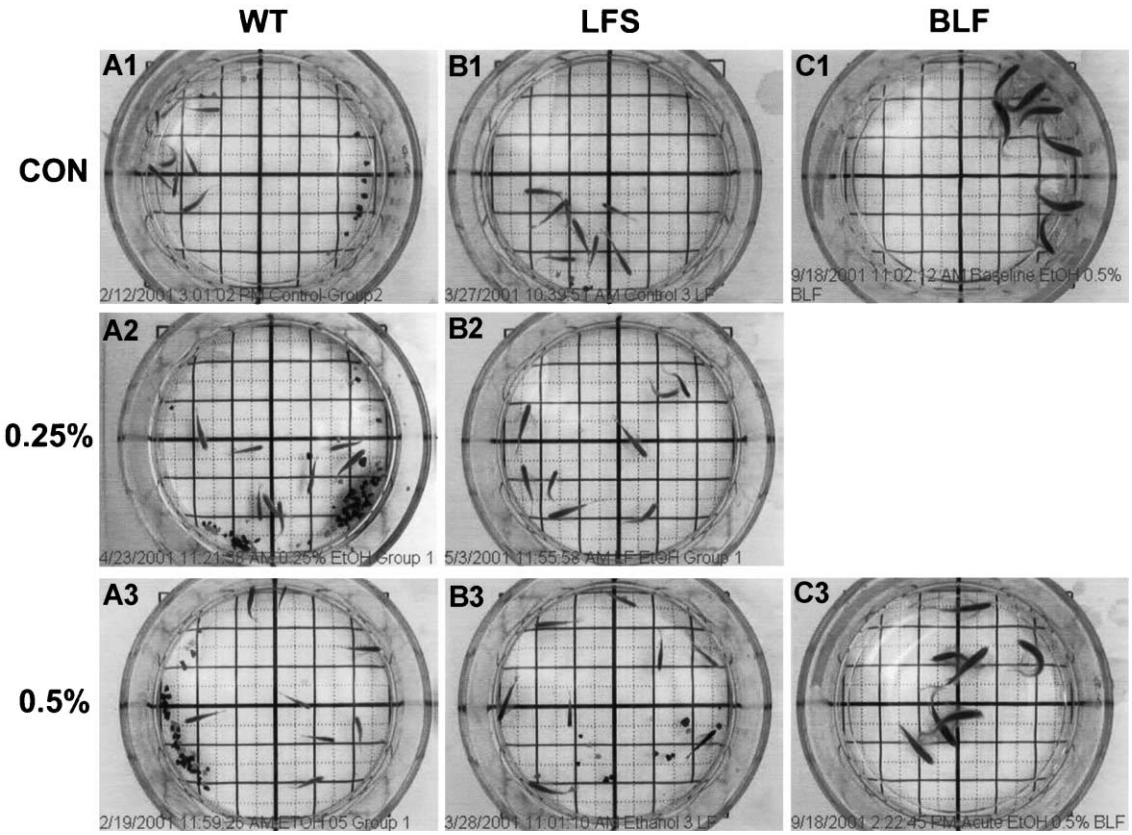

Fig. 1. Effect of various concentrations of ethanol on WT, LFS, and BLF zebrafish. Groups of WT, LFS, or BLF zebrafish were placed in a small tank with a 324-cm^2 grid and were exposed to various concentrations of ethanol (0, 0.25%, 0.5%, v/v). After 2 h, digital images of the fish were captured at 30-s intervals for 30 min using the system described in the Methods section. Shown are representative frames for each strain at the various concentrations of ethanol.

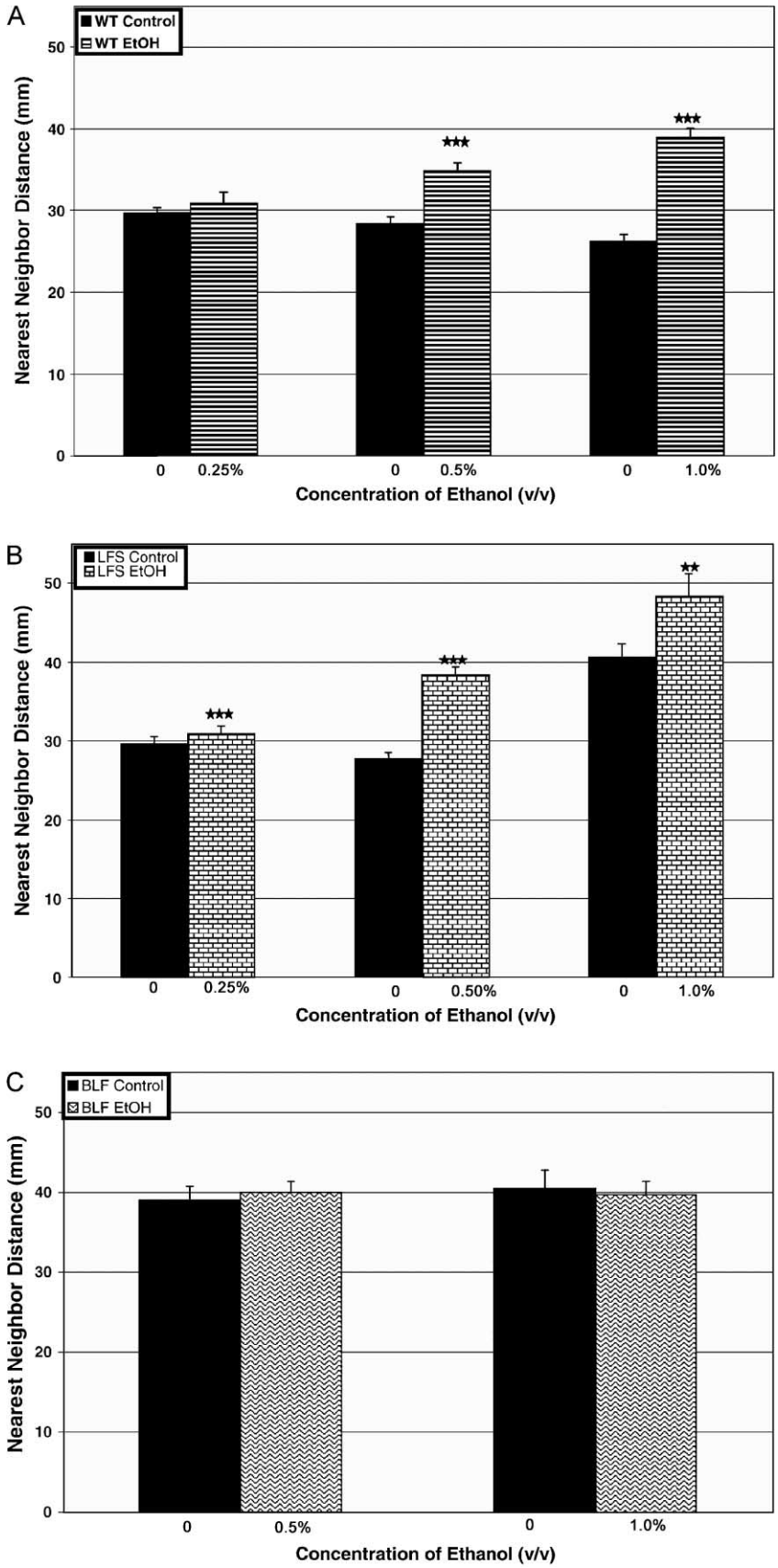

Fig. 2. Concentration-response relationships for the effect of acute ethanol on the average distance between nearest neighbors for (A) WT; (B) LFS; and (C) BLF zebrafish. After 2 h of exposure to various concentrations of ethanol (0, 0.25%, 0.5%, 1%, v/v), digital images of the groups of fish were captured at 30-s intervals for 30 min, and the distance in mm between each fish and its nearest neighbor determined as described in the Methods section. Control represents naive fish tested in the absence of ethanol. Data are plotted as mean ± S.E.M., $^{**}P < .01$, $^{***}P < .001$ compared to control.

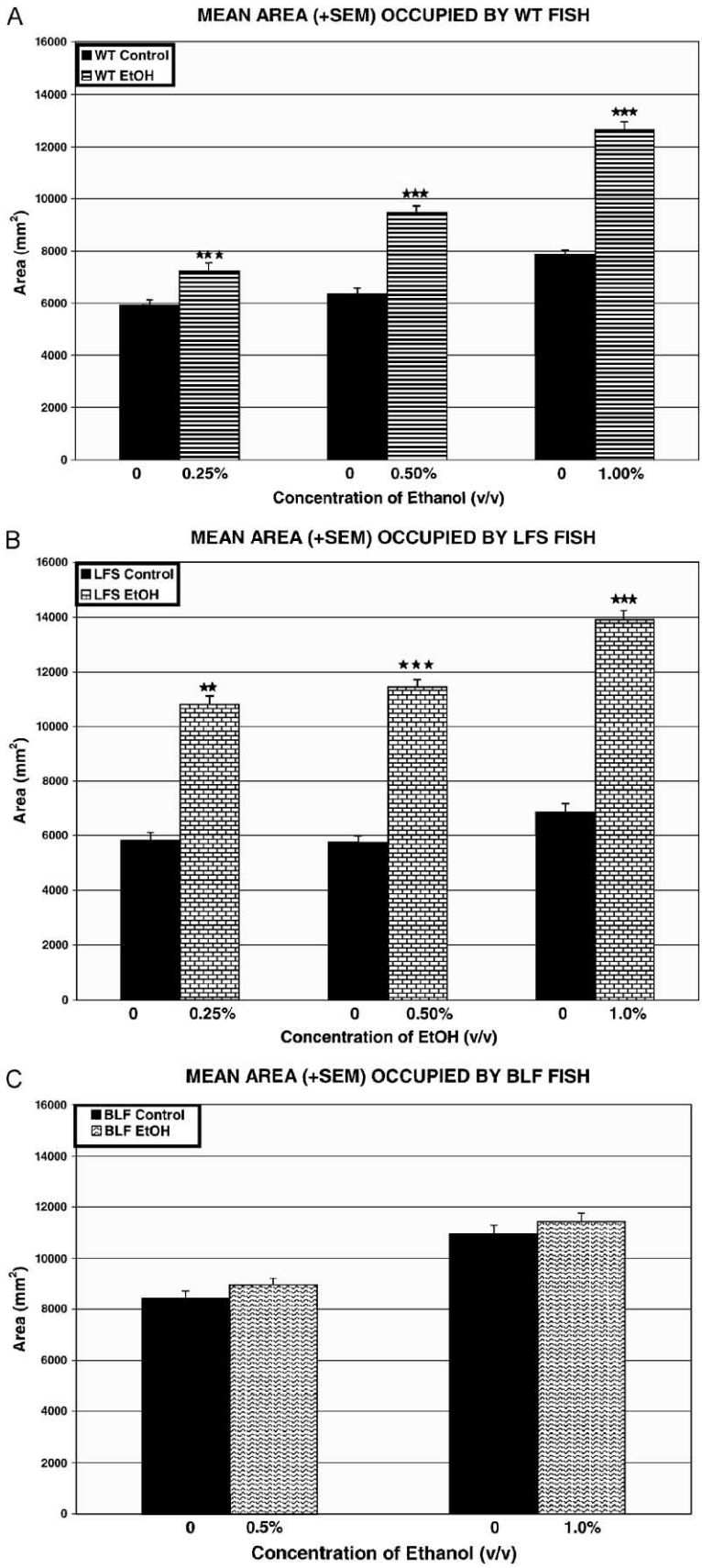

Fig. 3. The effects of various concentrations of acute ethanol on the total area occupied by (A) WT; (B) LFS; and (C) BLF strains of zebrafish. After 2 h of exposure to various concentrations of ethanol (0, 0.25%, 0.5%, 1%, v/v), digital images were captured at 30-s intervals for 30 min, and the total area occupied by the group of fish was estimated by measuring the area of the polygon formed by connecting the heads of the peripheral fish in each group as described in the Methods section. Control represents naive fish tested in the absence of ethanol on the same day. Data are plotted as mean ± S.E.M., $^{**}P < .01$, $^{***}P < .001$ compared to control.

detected with 0.25% (v/v) ethanol ($t = -3.196$, $df = 97$, $P < .001$) (Fig. 2B). There was also a significant increase in the area occupied by the LFS fish with 0.25% (v/v) ethanol ($t = -13.469$, $df = 253$, $P < .001$) (Fig. 3B).

Conversely, the BLF stain did not demonstrate ethanol-related modifications of swimming behavior (Fig. 1). In fact, in BLF fish, nearest neighbor distance ($t = -2.523$, $df = 41$, $P = .16$) (Fig. 2C) and the area occupied by each test group ($t = -1.032$, $df = 150$, $P = .304$) (Fig. 3C) were not increased with 1.0% (v/v) ethanol, the highest concentration of ethanol used in the study. Further analysis of the nearest neighbor distance with ANOVA using the factors of strain and ethanol concentrations of 0.5% and 1.0%, showed a significant interaction between strain and ethanol concentration [$F(2,191) = 7.882$, $P = .001$] due to a concentration-dependent increase in nearest neighbor distance in the LFS and WT strains while stable values, not significantly different from baseline, were observed within the BLF strain.

Acutely exposed WT and LFS treated fish also demonstrated a decreased startle reaction (Table 1). A decrease in the startle reaction was observed in LFS fish with 0.25% (v/v) ethanol ($t = 3.343$, $df = 29$, $P = .002$) and with 0.5% (v/v) ethanol ($t = 4.888$, $df = 23$, $P < .001$) in WT zebrafish (Table 1). Acute ethanol treatment did not affect the startle reaction in the BLF with concentrations as high as 1.0% (v/v) ($t = 0.298$, $df = 14$, $P = .770$).

Brain alcohol levels were measured to determine whether the above strain differences in the behavioral responses to ethanol had a pharmacokinetic basis. Within 15 min after the zebrafish were introduced to 0.5% (v/v) ethanol, significant alcohol levels were found in the brain (Fig. 4). With continuous exposure, brain alcohol content quickly reached a steady-state level that was maintained for at least 24 h. ANOVA using strain and time as factors showed that brain alcohol levels did not differ with time [$F(6,128) = 1.516$, $P = .178$] and strain [$F(2,128) = 1.143$, $P = .322$]. Furthermore, no interactions were present [$F(11,128) = 0.544$, $P = .87$].

Table 1
The mean (±S.E.M.) number of squares traversed by zebrafish when startled

Fish strain	Treatment ($n = 8$/group)	No. of squares traversed
Wild-type	Control	3.87 ± 0.51
	0.25% EtOH	3.42 ± 0.40
	Control	4.23 ± 0.32
	0.50% EtOH	1.58 ± 0.43***
	Control	3.75 ± 0.65
	1.0% EtOH	2.13 ± 0.55
Striped long-fin	Control	2.73 ± 0.25
	0.25% EtOH	1.63 ± 0.22 *
	Control	3.66 ± 0.48
	0.50% EtOH	1.94 ± 0.41 **
	Control	3.75 ± 0.49
	1.0% EtOH	1.57 ± 0.57***
Blue long-fin	Control	3.57 ± 0.20
	0.50% EtOH	2.71 ± 0.52
	Control	3.25 ± 0.37
	1.0% EtOH	3.00 ± 0.76

* $P < .05$, compared to controls.
** $P < .01$, compared to controls.
*** $P < .001$, compared to controls.

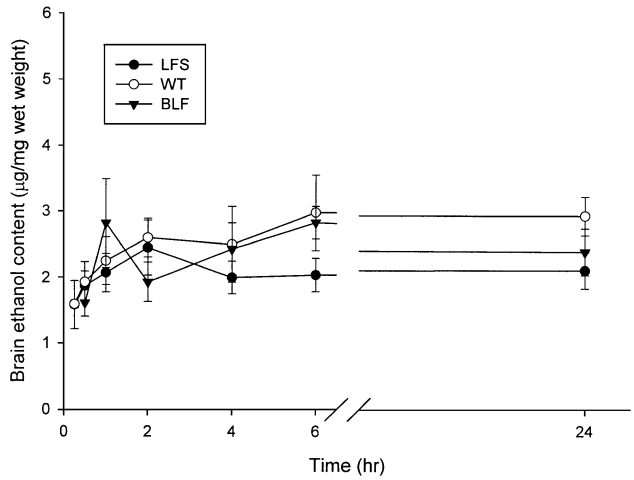

Fig. 4. Time course for brain alcohol levels. WT, LFS, and BLF zebrafish were treated with 0.5% (v/v) ethanol for up to 24 h. At various times, brains were rapidly removed, and alcohol content was measured as described in the Methods section. Data are expressed as micrograms ethanol/milligram wet weight of brain and are plotted as mean ± S.E.M. ($n = 4–9$ WT; $n = 5–9$ LFS; $n = 5–11$ BLF).

3.2. Chronic ethanol exposure

WT fish chronically exposed to 0.5% (v/v) ethanol swam in a less clustered pattern (Fig. 5A), whereas chronically treated LFS fish appeared more clustered (Fig. 5B). In the WT strain, the nearest neighbor distance was significantly longer than baseline measures [$F(2,69) = 23.869$, $P < .001$] at both 1 and 2 weeks of ethanol exposure as shown by the Tukey's post hoc test (Fig. 6). For comparison, the mean distance between WT zebrafish increased 23% with acute administration of 0.5% (v/v) ethanol (Fig. 2A) and 29–39% at 1 and 2 weeks of chronic ethanol exposure, respectively. Conversely, the nearest neighbor distance was not altered in ethanol-treated LFS fish relative to baseline [$F(2,73) = 2.391$, $P = .10$] (Fig. 6), suggesting the development of tolerance in the LFS group. ANOVA comparison of the two strains using treatment and strain as factors showed a significant interaction [$F(2,146) = 7.722$, $P = .001$] that further supports a genetic difference in the response to ethanol (Fig. 7). Differences in response to chronic ethanol treatment in the LFS strains were not related to mean brain alcohol levels in the LFS fish. Following 2 weeks of exposure to 0.5% (v/v) ethanol, the mean brain alcohol levels in the LFS fish was 1.86 ± 0.126 μg/mg brain ($n = 12$), a level not significantly different from acute brain alcohol levels in this strain. BLF fish were not affected by acute

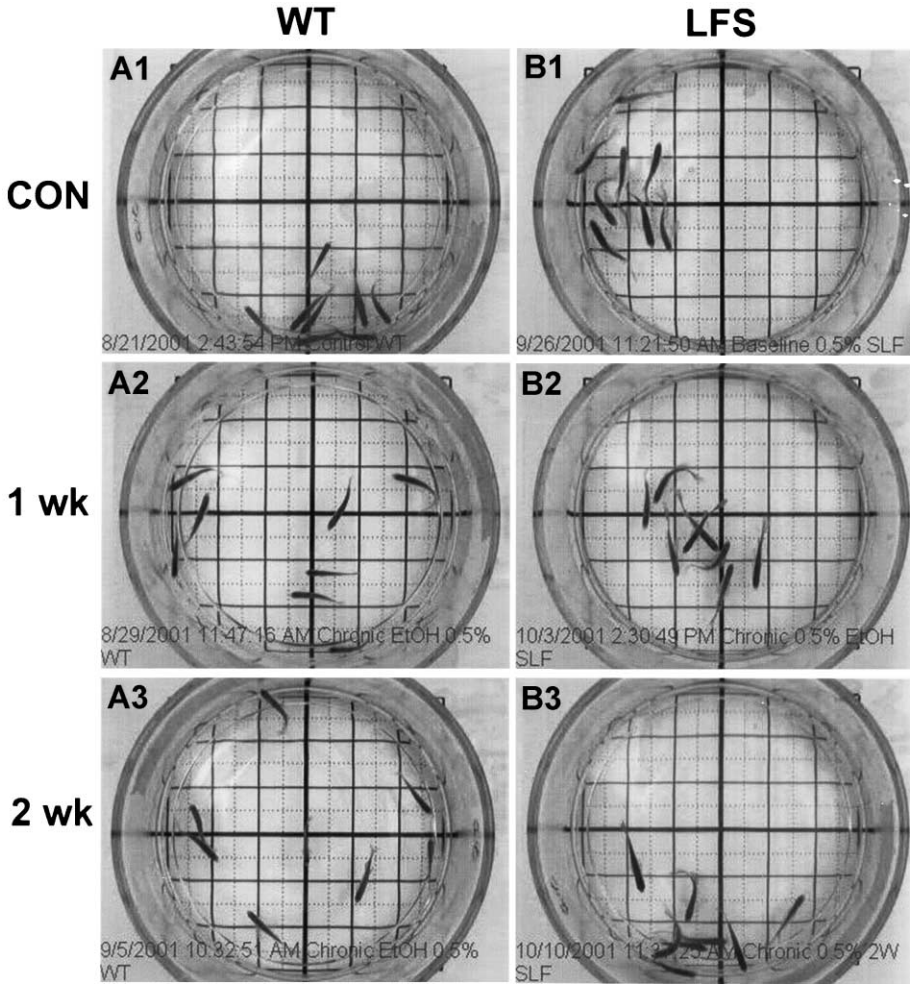

Fig. 5. Effect of chronic ethanol exposure on (A) WT and (B) LFS zebrafish. Groups of WT and LFS zebrafish were exposed to 0.5% (v/v) ethanol for up to 2 weeks. After 1 and 2 weeks of treatment, fish were transferred to a small tank that contained 0.5% (v/v) ethanol and was placed over a 324-cm^2 grid. Digital images of the fish were then captured at 30-s intervals for 30 min using the system described in the Methods section. Shown are representative frames prior to the initiation of the chronic ethanol exposure (i.e., baseline) and after 1 and 2 weeks of ethanol exposure.

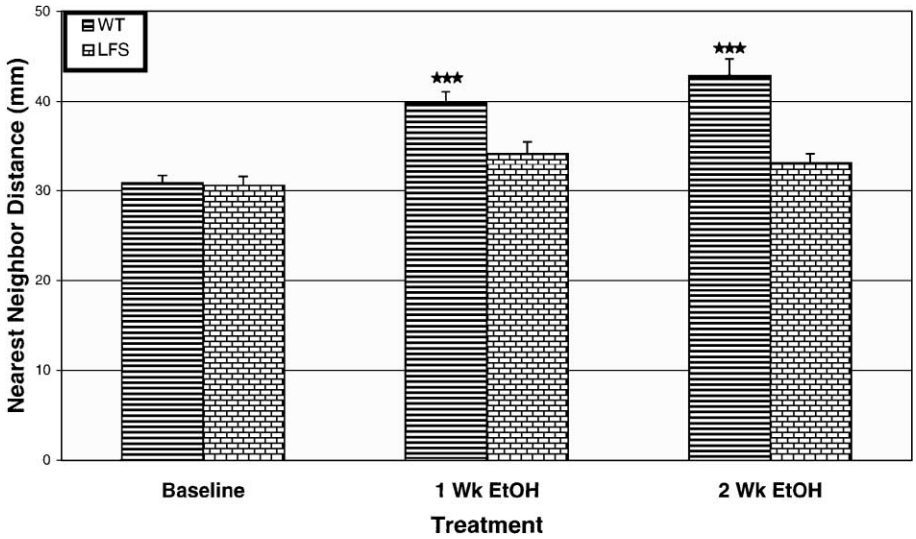

Fig. 6. Effect of chronic ethanol treatment on the average distance between nearest neighbors for WT and LFS zebrafish. After 1 and 2 weeks of treatment, fish were transferred to a small tank containing 0.5% (v/v) ethanol and placed over a 324-cm^2 grid. Digital images of the fish were then captured at 30-s intervals for 30 min, and the distance between each fish and its nearest neighbor was determined as described in the Methods section. Baseline represents data obtained on the group of fish prior to the start of the chronic ethanol treatment. Data are plotted as mean ± S.E.M., *** $P < .001$ compared to baseline.

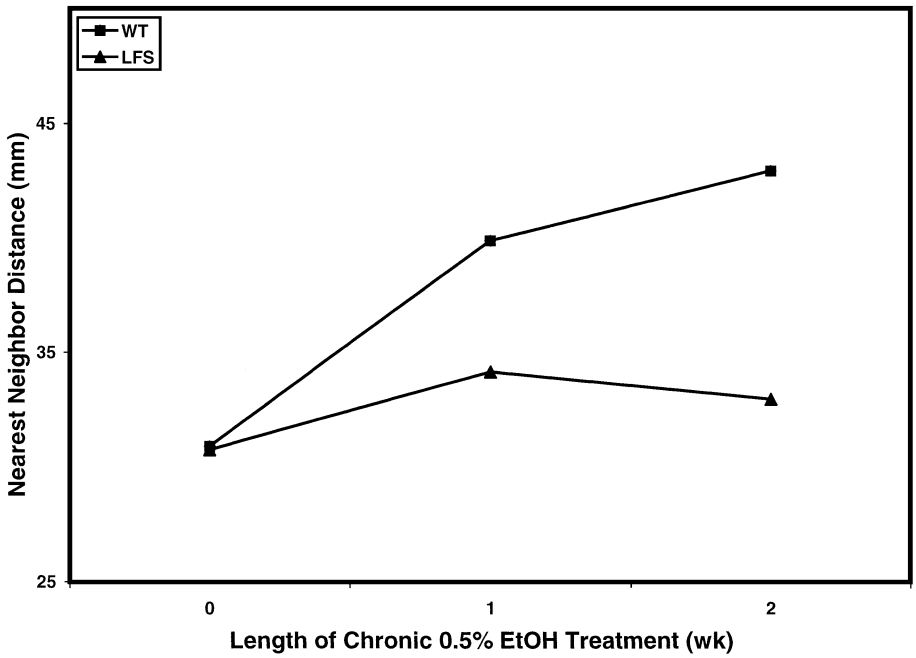

Fig. 7. Graphic representation of the significant interaction between the WT and LFS strain of zebrafish and the length of chronic 0.5% ethanol treatment. Baseline values for nearest neighbor distance (mm) were similar in the WT and LFS strains. During the 2-week course of ethanol treatment, nearest neighbor distance rapidly increased in the WT strain. In the LFS strain, however, there was a slight increase in nearest neighbor distance during the first week but nearest neighbor distance approximated baseline level at the end of the 2 weeks.

treatment with 1.0% ethanol, therefore, they were not tested chronically.

4. Discussion

The present study provides preliminary evidence that the zebrafish may be an excellent model for investigating the genetic determinants involved in response to ethanol. These data suggest that zebrafish behavior is altered by acute ethanol treatment and tolerance develops with chronic ethanol exposure. As has been shown in rodents (Phillips and Crabbe, 1991; Crabbe et al., 1994), the initial sensitivity to ethanol, as well as the development of tolerance, is influenced by the genotype of the subject. However, zebrafish are inexpensive and are easy to keep and breed. They have been extensively used for biomedical research, especially developmental studies, and accordingly, a large database of information is available on zebrafish (http://zfin.org/zf-info/dbase/db.html). As vertebrates, zebrafish are phylogenetically closer to mammals than are yeast, *C. elegans* or *Drosophila*. Significant genetic conservation has been shown between zebrafish and mammals (e.g., Inohara and Nuñez, 2000) and, thus, findings in zebrafish should be able to be extrapolated to mammals.

Fish readily absorb alcohol from their environment, and steady-state brain ethanol levels are rapidly achieved and maintained. In the present study, zebrafish brain alcohol levels were detected within 15 min of exposure, the earliest time point measured. Measurable brain alcohol levels, however, have been detected in goldfish after 2 min of exposure (Greizerstein and Smith, 1973). The present data are consistent with reports in other fish (Ryback et al., 1969; Greizerstein and Smith, 1973) in that, following a few hours of treatment, an equilibrium between the level of alcohol in the tank and the brain alcohol level of the zebrafish was achieved in which brain alcohol levels were approximately 90% of the tank alcohol level. Furthermore, with continual ethanol exposure, steady-state levels of brain alcohol could be maintained for at least a couple of weeks.

Zebrafish normally tend to swim as a group and stay in close proximity to each other as well as display a startle reaction to the rapid introduction of a novel stimulus. The former represents an inherent behavior of the fish that is easily quantified and can be measured nonintrusively. In the present study, these behaviors were used as indices to monitor for the effects of ethanol on the CNS. Acute exposure to ethanol increased the average distance between each fish and its nearest neighbor and inhibited the startle reaction. Similarly, Gerlai et al. (2000) also reported some ethanol-induced alterations in zebrafish behavior. In the present study, effects were dependent on the concentration of ethanol and also upon the strain of zebrafish. Thus, LFS zebrafish appear to be more sensitive to the acute effects of ethanol than the WT as the former consistently showed statistically significant effects with 0.25% ethanol (v/v) (i.e., 200 mg%, 43 mM). This concentration is approximately twice the legal limit for driving while intoxicated in humans and is comparable, if not less than, alcohol content reported in studies with rodents (Miller et al., 1980; Gatto et al., 1987; Wu et al., 2001).

Conversely, the BLF zebrafish appear to be fairly insensitive to the acute effects of alcohol as ethanol-induced alterations in behavior were absent with 1.0% ethanol (v/v) (i.e., 790 mg%, 171 mM). The difference in sensitivity of the various strains does not appear to reflect pharmacokinetic differences as brain alcohol levels were comparable among the three strains. Rather, the observed phenotypic differences in sensitivity appear to reflect a genetically determined difference in the response of the CNS to ethanol.

In the LFS zebrafish, the average distance between each fish and its nearest neighbor after 1 and 2 weeks of ethanol exposure was comparable to the pre-exposure baseline values. As 2 h of ethanol exposure significantly increased the average distance between fish, the lack of an effect after 1 week suggests that tolerance had developed in the LFS zebrafish. Tolerance can be classified as chronic, rapid, or acute based upon the rate of its development (LeBlanc et al., 1969, 1974; Gibbins et al., 1971; Crabbe et al., 1979; Bitrán and Kalant, 1991). Chronic tolerance, which was measured in the present study, can involve neuronal adaptation, changes in pharmacokinetics, or learning (Kalant et al., 1971). The chronic tolerance observed in the LFS zebrafish does not appear to entail an alteration in the pharmacokinetic properties of ethanol (e.g., increase in metabolism, change in distribution) as brain alcohol levels after 2 weeks of ethanol exposure were not significantly different from the levels observed after the acute treatment. Similarly, the procedure to treat and test the fish would preclude a role for "learning" in the observed development of chronic tolerance in the LFS zebrafish. Because ethanol was administered by maintaining the fish in an alcohol solution in their home tank, response expectancy (Vogel-Sprott and Sdao-Jarvie, 1989) and classical conditioning (Lê, 1979; Crowell et al., 1981; Melchior and Tabakoff, 1981), in which distinct environmental cues become associated with drug delivery and response, would not play a role in the development of tolerance. Similarly, the possibility of instrumental training (Wenger et al., 1981; Mansfield et al., 1983), whereby adaptation requires practicing the task while under the influence of the drug, is unlikely as a nonintrusive monitoring of typical zebrafish behavior, i.e., measuring distance between nearest neighboring fish, was used. Thus, the present study suggests that the observed chronic tolerance involved adaptation of the neurons in the CNS. Further, these results support a role for genetic determinants in regulating the development of chronic tolerance in zebrafish.

Interestingly, the WT zebrafish did not appear to develop tolerance. Rather, the average distance between each fish and its nearest neighbor appeared greater after 2 weeks of ethanol exposure compared to either the acute alcohol treatment or 1 week of ethanol exposure. The reason for this effect of chronic ethanol exposure in the WT zebrafish is unclear. It is possible and intuitively appealing to speculate that the chronic ethanol treatment was exerting a neurotoxic action. In support of this hypothesis, preliminary studies in the cerebellum of WT zebrafish show various ultrastructural alterations such as condensation of the chromatin material within granule cells and dilation of the smooth endoplasmic reticulum within Purkinje neurons dendrites in response to chronic ethanol treatment (Dlugos and Rabin, unpublished data). Similar ethanol-induced dilation of the smooth endoplasmic reticulum has been demonstrated in rodents after prolonged ethanol exposure (Dlugos and Pentney, 2000). The results do, however, support the findings that the genotype of the zebrafish influences the behavioral response to chronic ethanol treatment.

Zebrafish have a number of characteristics and properties that indicate they would be useful in identification of the genetic determinant involved in regulating the responses to ethanol. The results of the present study provide only preliminary information about the response of the zebrafish to ethanol but suggest that the zebrafish are sensitive to the effects of ethanol and that tolerance develops. In addition, these responses may be dependent upon the genotype of the zebrafish. Besides being inexpensive and easy to maintain and breed, zebrafish embryos are transparent and develop outside the body. Techniques for generating transgenic zebrafish are available (Meng et al., 1999) and with a few mating pairs large numbers of eggs can be collected daily for microinjection and production of transgenic animals. Thus, zebrafish could play a role as an inexpensive system to rapidly help identify the influence of various genes and gene products in regulating the effects of ethanol as well as an informational bridge between studies in invertebrates (e.g., Bellen, 1998; Moore et al., 1998; Scholz et al., 2000) with those in mammalian models.

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