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Short communication

Acute alcohol exposure increases tyrosine hydroxylase protein expression and dopamine synthesis in zebrafish

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HIGHLIGHTS

- Alcohol exposure increases dopamine and DOPAC.
- Alcohol exposure increases tyrosine hydroxylase expression.
- Alcohol exposure increases tyrosine hydroxlase activity.

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ABSTRACT

Zebrafish have become a popular animal model for investigating the effects of alcohol on the brain and behaviour. Acute exposure to alcohol has been shown to alter dopaminergic signalling in zebrafish, but the underlying mechanisms have not been well defined. In the current study, we characterize the effects of alcohol on the zebrafish dopaminergic system by focusing on tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis. Using western blot analysis, we demonstrate that a 60 min exposure to 1% alcohol increases tyrosine hydroxylase protein expression in the zebrafish brain. Enzymatic activity assays confirmed that alcohol also increases tyrosine hydroxylase enzymatic activity, whereas HPLC analysis demonstrated increased levels of whole-brain dopamine and its metabolite DOPAC. In addition to activation of the dopaminergic system, behavioural analysis revealed accompanying increase of distance traveled following 1% alcohol exposure. These findings suggest that acute alcohol exposure elevates dopamine synthesis via increased tyrosine hydroxylase protein expression. Our results support the hypothesis that alcohol alters dopaminergic signalling in the zebrafish brain in a similar manner as compared to mammals.

Alcohol (ethanol, ethyl alcohol) is known to produce a biphasic response depending on the concentration and duration of exposure. For example, ingestion of alcohol has been found to initially produce stimulant and euphoric effects with continued consumption leading to sedation and central nervous system depression [1]. Alcohol use disorders are prevalent and have an enormous impact on society in terms of costs associated with healthcare, crime, and lost productivity [2]. Individuals who are more sensitive to the stimulant effects of acute alcohol exposure have been found to be at a greater risk of developing alcohol addiction [3]. However, the mechanisms underlying alcohol's stimulant effect has not been completely understood.

Zebrafish have been a useful animal model for examining the neural mechanisms underlying alcohol's locomotor stimulant effect [4,5]. Acute exposure to low and moderate doses of alcohol increases locomotor activity in a dose-dependent manner quantified by the total distance traveled [6]. A number of studies have suggested the involvement of the dopaminergic system regulating alcohol induced locomotor activity in zebrafish. Stimulant doses of alcohol have been shown to increase whole-brain levels of dopamine and its metabolite 3, 4-dihydroxyphenylacetic acid (DOPAC) in zebrafish [7]. Furthermore, chronic alcohol exposure has been shown to attenuate acute alcohol challenge induced

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increase of whole-brain dopamine levels and locomotor activity, suggesting the involvement of the dopaminergic system in the development of tolerance to alcohol [7]. The involvement of the dopaminergic system in regulating alcohol induced locomotor activity is further supported by a recent study demonstrating that alcohol's locomotor stimulant effect is mediated by activation of dopamine D_2-like receptors in zebrafish [8].

The role of dopamine regulating alcohol induced locomotor activity has been heavily examined in the rodents (For review, see Ref. [9]). Although alcohol induced increase of whole-brain dopamine and DOPAC levels in zebrafish has been shown [4,7], the mechanism regulating this increase has only been recently examined [4,5]. Tyrosine hydroxylase (TH) is the rate-limiting enzyme responsible for dopamine synthesis, whereas monoamine oxidase is the enzyme responsible for the breakdown of dopamine into DOPAC [10]. A recent study found that alcohol increases the activity of TH without altering the activity of monoamine oxidase [11]. The measured increased overall activity of TH may be due to phosphorylation induced activation of the enzyme or to increased expression of the enzyme. A number of different kinases regulate the activity of TH in mammals [10]. Selective experimental inhibition of phosphorylated TH by tetrahydropapaveroline (THP) has been shown to inhibit dopamine synthesis in rodents [12] and zebrafish [4], confirming the role of phosphorylation of TH in dopamine synthesis in these distantly related species. Alternatively, alcohol exposure could increase TH activity following increased protein synthesis. A recent study found that alcohol exposure increased TH mRNA expression in the zebrafish brain [5]. However, alcohol induced TH protein expression changes have not been examined. In the current study, we characterize acute alcohol exposure induced changes in TH enzymatic activity, TH protein expression, dopamine and DOPAC levels and correlated alterations in behaviour.

81 adult (13 month old) zebrafish of mixed sexes were used for behavioural testing and tissue sample analysis. Zebrafish were housed in 37 L tanks (n = 20 per tank) with mechanical and biological filtration. Water quality parameters were monitored on a daily basis and maintained at optimal levels (pH: 6.5–7.5; conductivity: 250–500 μS; temperature: 27–29 °C).

Zebrafish were individually exposed to 0 or 1% v/v alcohol for 60 min in a 1.5 L trapezoidal tank filled with 1 L of water from their housing tanks (n = 40–41 per group). The back, bottom, and sides of the tank were covered with white corrugated plastic to minimize access external visual cues, and to provide a consistent testing environment. Video cameras were set up in front of each tank, and behavioural responses were recorded from the side view for the entire duration of the exposure. An automatic video tracking software (EthoVision XT 8.5) was used to analyze behavioural responses in the last 10 min of the recording. We chose to analyze behavioural responses during this time period since increases in brain alcohol concentration and dopamine levels have previously been shown to plateau during this period [7]. Furthermore, previous studies have suggested that zebrafish are able to habituate to the novel environment following this long exposure period [13]. For example, behavioural responses during the last 10 min of this exposure are less reflective of novelty-induced anxiety and more reflective of alcohol's effect on behaviour.

To examine changes in the dopaminergic system induced by acute alcohol exposure, zebrafish were immediately decapitated following the 60 min alcohol exposure period, and their brains were dissected and stored at −40 °C for future analysis. We quantified whole-brain tissue levels of dopamine and its metabolite DOPAC using high precision liquid chromatography (HPLC) employing a previously established protocol adapted for zebrafish [7]. Dopamine and DOPAC levels were standardized to brain protein content and reported as nmol per mg protein (n = 10 per group).

To examine changes in tyrosine hydroxylase activity following alcohol exposure, we quantified the enzymatic activity using a colorimetric activity assay according to a protocol previously adapted for zebrafish [11]. We also modified the enzymatic incubation period to account for the homeostatic temperature of zebrafish (28 °C). Tyrosine hydroxylase activity was standardized to brain protein content and reported as nmol L-DOPA produced per mg protein in 30 min (n = 10 per group).

To examine changes in the protein expression of tyrosine hydroxylase, we conducted a western blot for tyrosine hydroxylase using a previously established protocol adapted for zebrafish [14]. Anti-tyrosine hydroxylase antibody produced in rabbit (Sigma-Aldrich, Cat #T8700) at a dilution of 1:1000, HRP-conjugated anti-rabbit antibody (Sigma-Aldrich, Cat #A6154) at a dilution of 1:5000, and HRP-conjugated anti-β-actin antibody (Sigma-Aldrich, Cat #A3854) at a dilution of 1:20,000 were used for immunoblotting. Tyrosine hydroxylase expression was reported relative to β-actin (n = 6 per group).

The data were analyzed using IBM SPSS 21 for windows. Different groups (0 and 1% alcohol) were compared using independent sample *t*-tests with significance reported at $p \leq 0.05$.

Fig. 1 shows the effects of acute alcohol exposure on different behavioural responses. Exposure to 1% alcohol increased the total distance traveled ($t = 2.288$, $df = 79$, $p = 0.025$; Fig. 1A), and decreased variance of distance to bottom ($t = 2.345$, $df = 79$, $p = 0.022$; Fig. 1B) without altering absolute turn angle ($t = 1.019$, $df = 79$, $p = 0.311$; Fig. 1C) and distance to bottom ($t = 0.443$, $df = 79$, $p = 0.659$; Fig. 1D) in the last 10 min of exposure.

Fig. 2 shows the effects of acute alcohol exposure on the levels of whole-brain dopamine and its metabolite, DOPAC. Quantification of these neurochemicals in the zebrafish brain revealed that alcohol exposure increased whole-brain levels of dopamine ($t = 4.807$, $df = 18$, $p < 0.001$; Fig. 2A) and DOPAC ($t = 3.228$, $df = 18$, $p = 0.005$; Fig. 2B). To examine changes in dopamine synthesis, we quantified the enzymatic activity and protein expression of tyrosine hydroxylase (Fig. 3). Enzymatic activity assays revealed that alcohol increased the activity of tyrosine hydroxylase in whole-brain homogenates ($t = 2.633$, $df = 18$, $p = 0.017$; Fig. 3A). Western blot analysis revealed that alcohol increased the expression of tyrosine hydroxylase relative to β-actin ($t = 4.432$, $df = 10$, $p = 0.001$; Fig. 3B).

Alcohol induced behavioural changes in zebrafish have previously been reported in the literature [15,16]. Our behavioural results show that alcohol increased the total distance traveled and decreased the variance of distance to bottom, which is in line with previous findings [6,8]. The behavioural results suggest that alcohol exposure altered motor responses by making fish significantly more active and less likely to change their vertical position in the tank. Alcohol's locomotor stimulant effect (quantified by an increase in total distance traveled) is often examined in relation to the dopaminergic system both in mammals [9,17] and in zebrafish [5,8,18]. Previous studies have found that alcohol exposure increases whole-brain dopamine levels [7] as well as tyrosine hydroxylase mRNA expression [5] in zebrafish, suggesting an important role for this rate-limiting enzyme. In the current study, we show for the first time that a 60 min exposure to 1% alcohol increases whole-brain tyrosine hydroxylase protein expression in zebrafish. In addition, we also report that alcohol exposure increases both the enzymatic activity of tyrosine hydroxylase, as well as whole-brain levels of dopamine and its metabolite DOPAC. Our findings suggest that alcohol exposure in zebrafish increases the synthesis of whole-brain dopamine by increasing tyrosine hydroxylase activity via increased protein expression.

Nowicki et al. [4] previously reported that acute alcohol exposure induced increases in whole-brain dopamine is dependent on tyrosine hydroxylase phosphorylation in zebrafish. Our results now show that the alcohol induced dopamine level increase is