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Individual differences in response to alcohol and nicotine in zebrafish: Gene expression and behavior

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Abstract

Alcohol and nicotine are psychoactive substances responsible for serious health consequences. Although the biological mechanisms of alcohol and nicotine have been studied extensively, individual differences in the response to these drugs have received little attention. Here we evaluated gene expression and behavior of bold and shy individuals after acute exposure to alcohol and nicotine. For this, zebrafish were classified as bold and shy individuals based on emergence tests, and then fish were exposed to 0.00, 0.10, and 0.50% alcohol or 0.00, 1.00, and 5.00 mg/L nicotine and their anxiety-like and locomotor behavior was observed. After behavioral assessment, brain mRNA expression (*ache*, *bdnf*, *gaba1*, *gad1b*, *th1*, and *tph*1) was evaluated. Locomotion patterns differed between profiles depending on alcohol and nicotine concentration. Anxiety increased in shy fish and decreased in bold fish after exposure to both drugs. Alcohol exposure induced an increase in *tph1* mRNA expression in bold fish, while *bdnf* mRNA expression was increased in shy fish. Nicotine increased *ache*, *bdnf*, and *tph1* mRNA levels in both profiles, but at higher levels in bold fish. Based on our research, we found that alcohol induces anxiogenic effects in both bold and shy zebrafish. Additionally, shy individuals exposed to a low concentration of nicotine exhibited stronger anxiety-like responses than their bold counterparts. These findings further support the validity of using zebrafish as a dependable tool for studying the effects of drugs and uncovering the underlying mechanisms associated with individual variations.

KEYWORDS
acetylcholine, anxiety, boldness, dopamine, personality, serotonin

1 | INTRODUCTION

Individual differences have been studied in a variety of species and explored in recent decades due to the correlation between traits and the ability to make decisions (Wang et al., 2015), deal with stress (Carere et al., 2005), overcome illness (Cavigelli, 2005), and develop addiction (Goldman et al., 2005). Although behavioral traits are plastic and vary depending on physiological and genetic factors, some responses are consistent over time and across contexts (Koolhaas et al., 1999; Øverli et al., 2007; Wilson et al., 1994). Some behavioral characteristics that are correlated are usually grouped into sets, which are referred to as temperament (Réale et al., 2007), personality (Gosling & John, 1999), coping style (Koolhaas et al., 1999), behavioral

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syndrome (Sih et al., 2004), and behavioral profile (Svartberg, 2005). The response to novel and stressful situations is one of the most extensively investigated among the various behavioral features. This particular aspect is often called the "boldness axis" or "boldness–shyness continuum" (Oswald et al., 2012).

Bold individuals tend to take more risks; they habituate faster, are more apt to explore novelty, and have their behavioral responses more linked to routines and previous experiences (White et al., 2017). Shy individuals adopt a risk-averse strategy, have low exploration rates, demonstrate greater behavioral flexibility, and are highly dependent on ambient signals (Moscicki & Hurd, 2015; Wilson et al., 1994). In fish, bold individuals are more aggressive (Øverli et al., 2004), are more active (Ferrari et al., 2015; Millot et al., 2009), and depict stronger sympathetic reactions and lower hypothalamic–pituitary–interrenal (HPI) reactivity than shy ones (Alfonso et al., 2019; Huntingford et al., 2010). The bold–shy dimension includes an individual's inclination to explore potentially risky situations (Stamps, 2007). Consequently, individuals classified as bold are anticipated to have a greater propensity for drug use and abuse, often display diminished fear and anxiety responses, leading to a higher willingness to take risks, and experiment with substances that others may consider hazardous (Araujo-Silva et al., 2018; Bellot et al., 2022; Daniel & Bhat, 2020; Kanai & Rees, 2011; Mamuneas et al., 2015). This combination of personality traits and behaviors heightens their vulnerability to drug use and raises the risk of developing substance abuse or dependence.

The susceptibility to alcohol and nicotine abuse differs between individuals, and several mechanisms contribute to it, including genes that regulate some brain neurotransmitters, changes in drug responsiveness as tolerance and sensitization, conditioning (the search for the drug elicited by certain signals), and psychosocial factors that include personality (Bahi, 2013; Bobo & Husten, 2000; Breslau, 1995; Goldman et al., 2005; Wolf et al., 2007). While the biological mechanisms of alcohol and nicotine have been studied extensively and serious health consequences are attributed to their use, individual differences in the response to these drugs received little attention. The literature on this topic is still limited and there is a gap in the knowledge of the relationship between the propensity to develop addiction, neurophysiological changes, and differences in the behavioral profile of individuals. A few recent studies investigated the relationship between behavioral characteristics and alcohol or nicotine consumption in zebrafish (Araujo-Silva et al., 2020; Klee et al., 2011), an animal model for translational research that has been contributing to the thorough understanding of several human complex diseases (Xu & Zon, 2010).

In both human and animal models, chronic exposure to alcohol and nicotine may lead to the development of tolerance and can increase the risk of addiction (Elvig et al., 2021; Koob, 2014; Wills & Kenny, 2021). These substances stimulate the release of dopamine, a neurotransmitter associated with reinforcing effects and the experience of pleasure (Klee et al., 2012; Koob & Colrain, 2020; Roberto et al., 2010). These drugs have an impact on the serotonergic pathways, which are integral to the regulation of emotional well-being and mental health. The serotonergic pathways play a vital role in modulating mood and influencing the manifestation of anxiety-like responses (Johnson, 2004; Mathur & Guo, 2010).

In terms of behavior, acute alcohol exposure has a biphasic action: low to moderate doses cause stimulant and anxiolytic effects, whilst high doses promote depressant effects (Müller, Ziani, et al., 2020; Tran et al., 2016). Alcohol potentiates γ-aminobutyric acid (GABA) transmission and inhibits glutamatergic transmission, causing an initial relaxing sensation followed by changes in locomotor activity, impairments in environment perception and attention, and damage to cognitive performance (Banerjee, 2014; Davies, 2003). Concerning nicotine's mechanism of action, the stimulant activity of this drug in the central nervous system modulates and mediates behavioral and cognitive effects (Sherafat et al., 2021). Nicotine acts as an agonist to the cholinergic system by binding to nicotinic cholinergic receptors (nAChRs) (Tarren et al., 2016).

The repeated use of alcohol and nicotine has the acute effects described above. The continued use of the drug, chronic or repeated, may generate a situation of tolerance in which the individual starts to need higher concentrations of the drug to achieve the same effects (Benowitz, 2010; Koob & Volkow, 2010). In the long term, dependence can develop; however, this propensity varies from individual to individual. These responses have been reported to correlate between human beings and animal models such as the zebrafish (Araujo-Silva et al., 2018; Echevarria et al., 2011; Kalueff et al., 2014; Mathur & Guo, 2010).

In this sense, the neurobiological mechanisms of both alcohol and nicotine were largely studied, but the reasons for different outcomes observed between individuals are still obscure. While some individuals hardly become dependent, others start craving for the drug after a few opportunities of use (Cui et al., 2012; Hendrickson et al., 2013). To understand the susceptibility of individuals to become addicted to drugs, a few researchers have considered personality aspects (Skóra et al., 2020; Wingo et al., 2016). For instance, considering parameters in the bold–shy dimension, risk-prone and risk-averse individuals show lower and higher sensibility to drug effects, respectively (Araujo-Silva et al., 2020; Eddins et al., 2009). Aiming to fill this gap in the literature, the objective of this work was to evaluate how bold and shy zebrafish react to alcohol and nicotine in terms of behavior and gene expression. We developed a novel tank including areas of refuge, exploration, and risk taking and evaluated how bold and shy individuals react after being exposed to increasing concentrations of alcohol and nicotine. We hypothesized that the bold profile would be less affected by the drugs while shy individuals would be more sensible.

2 | MATERIALS AND METHODS

2.1 | Ethical note

The Animal Use Ethics Committee of the Federal University of Rio Grande do Norte analyzed and approved all fish maintenance and experimental protocols of this study (CEUA, certificate number

263.033/2021). Animals' health and well-being were monitored daily during all experimental phases. In all phases of the present study, the authors complied with the ARRIVE guidelines.

2.2 | Animal husbandry

Adult zebrafish (*Danio rerio*, wild-type strain, final $N = 120$, 6 months old, female:male ~ 1:1, 0.58 ± 0.11 g) were obtained from a local farm (Natal-RN, Brazil) and housed for 3 months in the fish laboratory of the Federal University of Rio Grande do Norte under the following conditions. The fish were stocked in an automated racking system (28 cm × 11 cm × 18 cm, width × depth × height; 5.5 L, ZebTEC Active Blue—Tecniplast®) with a density of 10 fish per tank and kept at 28°C (± 1°C) with a 14 h light/10 h dark photoperiod (250 lux). Water osmolality (600 μS) and pH (6.7) were automatically controlled. Animals were fed twice a day, in the morning with nauplii of *Artemia salina* (Artemia salina do RN®, Brazil) and in the afternoon with flake food (Alcon Basic® 60% protein and 15% fat).

2.3 | Behavioral profile determination

Behavioral profiles were established according to the protocol described by Tudorache et al. (2013), based on the tendency of zebrafish to prefer dark areas and to remain close to the social group. For the emergence test, the tanks (40 cm × 25 cm × 20 cm, width × depth × height; 20 L) were filled up to 15 L and divided by an opaque partition into two equal-sized compartments: a black side with black walls (initial zone) and a white side with white walls. The middle partition had a guillotine door to control fish transition to sides, as described by Araujo-Silva et al. (2018). All water and laboratory conditions were the same as described above.

The emergence test was carried out twice. In order to select an initial group of bold and shy animals, 670 fish were used. Among these 670 fish, 200 were categorized as shy and another 200 as bold. Only those displaying early emergence behavior were retained for the bold category, while only those exhibiting late emergence were kept for the shy category. This was the first round of separation. Afterwards, a second round was performed: 60 bold animals were selected among the 200 pre-selected bolds, and 60 shy animals were selected among the 200 pre-selected shy animals, resulting in a final sample of 120 animals. After the second round of emergence testing, the animals were returned to their respective stock tanks. All animals identified as having intermediate characteristics during the first and second rounds of emergency separation were excluded from further analysis.

The procedure for carrying out the emergence test in the two separation rounds described above was as follows: fish were randomly selected from the stock tanks and divided into groups of 10 individuals that were placed together on the black side of the tank. After a habituation period of 2 min, the guillotined door was raised, and fish could cross to the white side. Every time a fish crossed the door, it was closed, and the animal was removed from the tank. Then, the door was opened again for the next fish. The first three fish to emerge to the white part were considered bold and the last three were considered shy. The remaining four fish were named intermediates and were not used in this study. This procedure was repeated with all groups.

2.4 | Drug exposure and behavioral registration

Drug exposure lasted 60 min for alcohol (99.9% absolute ethyl alcohol, Dinâmica Química Contemporânea Ltda, Brazil) and 3 min for nicotine ((−)-nicotine ≥99% (GC) liquid, Sigma-Aldrich, Brazil, CAS Number: 54-11-5), according to Miller et al. (2013). The animals were divided into 12 groups: two of bold animals ($n = 10$ per group) and two of shy animals ($n = 10$ per group) treated with alcohol (concentration 0.10% and 0.50%); two of bold animals ($n = 10$ per group) and two of shy animals ($n = 10$ per group) treated with nicotine (concentration 1.00 mL/L and 5.00 mg/L). The same control group was used for both drugs ($n = 10$ for the bold control group; $n = 10$ for the shy control group). Shortly after acute exposure, fish were individually filmed in a ramp aquarium adapted from Walsh-Monteiro et al. (2016) to analyze the exploratory behavior.

The animals were individually transferred to a tank that had an inclined ramp (40 cm × 10 cm × 20 cm; 23° of inclination) as shown in Figure 1. The non-tilted part of this tank, called the flat area (10 cm length), was covered with black adhesive paper and was the initial zone where the fish were placed individually. The sloped part of the tank, called the ramp area (20 cm length), was covered with opaque white adhesive paper. At the top of the ramp there was a cubic object (3 cm^2, green color, Lego®) called the object area (10 cm length). The behavior was recorded individually for 10 min with a webcam (Logitech c920 HD Pro) positioned in front of the tank and was analyzed by ZebTrack/UFRN (Pinheiro-da-Silva et al., 2017). The parameters evaluated were time spent in each area, distance from the object, average speed while moving, total distance traveled, distance from the bottom of the tank, and time immobile (time stopped in absence of movement).

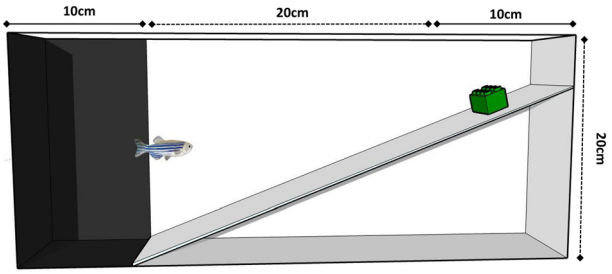

FIGURE 1 Overview of the exploration tank with ramp (40 cm x 10 cm x 20 cm; 23° of inclination), showing the flat area (black), the ramp area, and the object area. The whole bottom of the tank was completely covered with opaque self-adhesive plastic films (white or black). At the top of the ramp, there was a green cubic object (3 cm^2).

2.5 | Molecular analysis

After behavioral registration, the animals were euthanized in clove oil (200 mg/L, eugenol-based anesthetic), and brain tissue was collected for gene expression analysis. Removed brains were grouped in two pools with five brains in each tube containing 1 mL of RNA-Later (Sigma-Aldrich®) and stored at −20°C according to the RNA-Later process. Total RNA was extracted using the PureLink® RNA Mini Kit according to the manufacturer's instructions. The purity and quality of extracted RNA were analyzed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, CA, USA) with an A_{260}/A_{280} ratio between 1.8 and 2.0, and the quality was analyzed by electrophoresis in a 0.8% agarose gel. Reverse transcription reactions were performed using the TaqMan Reverse Transcriptase Reagent kit with 1 µg of total RNA.

qPCR analysis was performed using the Rotor-gene Q system (Qiagen, CA, USA) and Power SYBR® Green PCR Master Mix. Reactions were performed with 5 µM of the chosen primer pair, 1× SYBR Green PCR Master Mix, 2 µL of template cDNA, and UltraPure™ Nuclease-Free Distilled Water to reach a final volume of 20 µL. The running conditions were as follows: a 10-min denaturation step at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s, a melting curve stage at 95°C for 15 min and 60°C for 1 h, and a final step of 15 min at 95°C. The primers were selected based on targets of alcohol and nicotine neurotransmitter systems and prior studies of zebrafish gene expression in response to alcohol and nicotine exposure (Table 1). Analysis of mRNA expression was performed following the Minimum Information for Publishing Quantitative Real-Time PCR Experiments (MIQE) guidelines.

2.6 | Data analysis

Data were analyzed for homogeneity, normality, collinearity, and possible outliers, as suggested by Zuur et al. (2010). Then, we conducted two-way analysis of variance (ANOVA) followed by Bonferroni post hoc correction to evaluate the main effect of profile (two levels: bold and shy) and alcohol or nicotine treatment (three levels: 0.00%, 0.10%, and 0.50% or 0.00, 1.00, and 5.00 mg/L, respectively). The test was used to analyze distance from the object, average speed while moving, total distance traveled, distance from the bottom of the tank, and time immobile. In order to examine the variations in the time spent in different areas of the tank between groups, we conducted three-way ANOVA. This analysis was followed by post hoc comparisons using Bonferroni correction. The purpose was to explore the main effects of alcohol/nicotine exposure (with three levels in each treatment), behavioral profile (bold and shy), and tank area (with three levels: flat, ramp, and object). Additionally, we investigated the interactions among these factors. Data were analyzed using the "tidyverse," "rstatix," "ggpubr," and "emmeans" packages (Kassambara, 2020a; Kassambara, 2020b; Lenth & Lenth, 2018; Wickham et al., 2019) in the R program (R-Studio version 4.0.1 for Windows). Differences were considered significant if $p \leq 0.05$ (*$p \leq .05$, **$p \leq .01$, ***$p \leq .001$, ****$p \leq .0001$).

TABLE 1 Genes, functions, and primer sequences for all target and reference genes examined in quantitative real-time PCR analysis.

Gene	Function	Associated neurotransmitter	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)	Database accession number
Acetylcholinesterase (*ACHE*)	Converts acetylcholine into choline and acetate	Acetylcholine	GGCGAAGAGCGGACGAATATC	AAGGAGACCATTCAGCAGGACAG	NM_131595.2[a]
Tyrosine hydroxylase (*TH1*)	Converts tyrosine into L- DOPA	Dopamine	GACGGAAGATGATCGGAGACA	CCGCCATGTTCCGATTTCT	NM_178306.3[a]
Tryptophan hydroxylase (*TPH1*)	Converts tryptophan into 5-hydroxytryptophan	Serotonin	CAGTTCAGTCAGGAGATTGG	GACAGTGCGTGCTTCAG	XM_017354058.2[a]
Glutamate decarboxylase (*GAD1B*)	Synthesis of GABA through decarboxylation of glutamate	GABA	CATACGCACAATACGCTGCC	TACACAGCACCATGCGAGTT	NM_131846.2[a]
Gaba receptor (*GABA1*)	$GABA_A$ receptor	GABA	AAGAGCCAAAACCCAAACCT	CCTTTACAGACACGGCCATT	NM_001077326.1[a]
Brain-derived neurotrophic factor (*BDNF*)	Neuroprotection, neuronal growth, and regeneration	BDNF	AACTCAGGCGATTGTTGCAT	TGAGGACATTTCCAGCCTTC	NM_194419.1[a]
β-Actin	Endogenous control	-	CTGTTCCAGCCATCCTTCTT	TGTTGGCATACAGGTCCTTAC	NM_181601.5[a]

[a]NCBI database (available at https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi).

3 | RESULTS

3.1 | Behavioral responses

The total time spent in each area of the tank is presented in Figure 2. For the fish exposed to alcohol (Figure 2a), three-way ANOVA revealed significative effects of areas ($F_{2,532} = 83.18$, $p < .001$), but no effects of alcohol ($F_{2,532} = 0.001$, $p = .99$) and profile ($F_{1,532} = 0.001$, $p = .97$). The interaction terms alcohol × areas ($F_{4,532} = 20.75$, $p < .001$) and alcohol × areas × profile ($F_{4,532} = 4.35$, $p = .002$) were found significant, but the interaction terms profile × area ($F_{2,532} = 2.77$, $p = .06$) and profile × alcohol ($F_{2,532} = 0.002$, $p = .99$) were non-significant. The Bonferroni post hoc test showed that bold 0.10% and bold 0.50% spent more time in the flat area than bold 0.00%, and shy 0.50% spent more time in the flat area than shy 0.00%. Regarding time spent in the ramp area, bold 0.00% differed from bold 0.10% and bold 0.50%, while shy 0.00% differed from shy 0.50% ($p < .05$).

For the animals treated with nicotine (Figure 2b), three-way ANOVA showed significant effect of areas ($F_{2,560} = 62.48$, $p < .001$), but no effect of nicotine ($F_{2,560} = 0.001$, $p = .99$) and profile ($F_{1,560} = 0.001$, $p = .96$). The interaction terms nicotine × areas ($F_{4,560} = 20.32$, $p < .0001$), areas × profile ($F_{4,560} = 3.93$, $p = .02$), and nicotine × areas × profile ($F_{4,560} = 2.49$, $p = .04$) were found significant. The Bonferroni test showed that bold 1.00, bold 5.00, shy 1.00, and shy 5.00 spent more time in the flat area than the control animals (bold and shy 0.00). Therefore, bold and shy 0.00 animals spent more time in the ramp area ($p < .05$). The interaction term nicotine × profile ($F_{2,560} = 0.002$, $p = .99$) was non-significant.

Figure 3 shows the average distance from the object. For alcohol treatment groups (Figure 3a), object distance varied between treatments and two-way ANOVA revealed statistical significance for alcohol treatment ($F_{2,53} = 11.94$, $p < .001$). No significant effect was observed for profile ($F_{1,53} = 0.11$, $p = .73$) and the interaction term alcohol treatment × profile ($F_{2,53} = 1.24$, $p = .2$). The Bonferroni test showed that the bold 0.10% and 0.50% and shy 0.50% groups had a higher mean object distance than the bold and shy 0.00% groups ($p < .05$). For the zebrafish exposed to nicotine (Figure 3b), two-way ANOVA indicated no significant effects of the profile ($F_{1,49} = 2.24$, $p = .14$) or the interaction term nicotine treatment × profile ($F_{2,49} = 2.17$, $p = .12$), but statistical significance was observed for nicotine treatment ($F_{2,49} = 7.74$, $p = .001$). The Bonferroni test revealed that the shy 1.00 and 5.00 groups kept more distance from the object than the other groups ($p < .05$).

Figure 4 shows other locomotor parameters (total distance traveled) and anxiety-like behaviors (distance from the bottom of the tank and time immobile) of zebrafish after alcohol and nicotine exposure. Regarding alcohol exposure, for total distance traveled, two-way ANOVA showed a significant effect of alcohol ($F_{2,53} = 1.18$, $p < .0001$), but no significant effects of profile ($F_{1,53} = 43.43$, $p = .66$) and the interaction term profile × alcohol ($F_{2,53} = 1.80$, $p = .17$). The post hoc test also indicated that bold 0.00% differed from bold 0.10% and 0.50%, and shy 0.00% differed from shy 0.10% and 0.50% ($p < .01$; Figure 4a). Regarding distance from the bottom of the tank

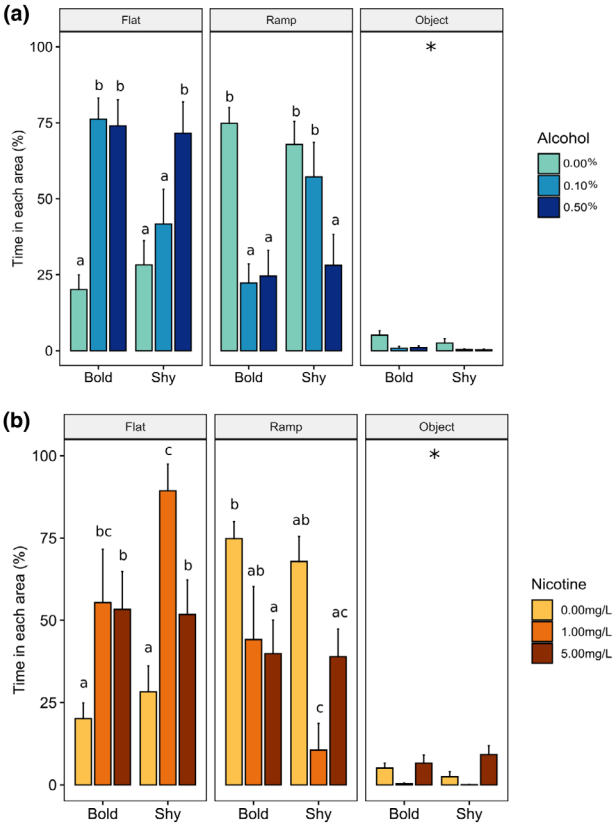

FIGURE 2 Zebrafish behavior in the exploration tank with ramp after (a) alcohol or (b) nicotine treatment. The experimental tank was divided into three areas: a flat area, a ramp area, and an object area located at the top of the ramp. Fish were acutely treated with alcohol (60 min, $n = 10$ per group) or nicotine (3 min, $n = 10$ per group) and then placed in the ramp tank (flat area). Behavior was recorded for 10 min. Results are presented as mean ± SEM. Different letters indicate statistically significant differences between treatments in each area (bold and shy groups: three-way ANOVA, $p < .05$). Asterisks indicate statistically significant differences between areas ($p < .05$).

(Figure 4b), two-way ANOVA showed that there was no statistical significance for behavioral profiles ($F_{1,53} = 3.32$, $p = .07$) and the interaction profile × alcohol ($F_{2,53} = 1.03$, $p = .36$). However, statistical significance was observed for alcohol concentrations ($F_{2,53} = 8.81$, $p = .0004$). Bold and shy 0.50% differed in distance from the bottom, and shy 0.50% was closer to the bottom of the tank. For time immobile (Figure 4c), two-way ANOVA revealed no statistical significance of profiles ($F_{1,53} = 0.22$, $p = .63$) and of the interaction profile × alcohol ($F_{2,53} = 1.64$, $p = .20$), but statistical significance was observed for alcohol treatment ($F_{2,53} = 46.04$, $p = .0001$). The Bonferroni post hoc test revealed that bold 0.00% differed from bold 0.10% and 0.50%, and shy 0.00% differed from shy 0.10% and 0.50% ($p < .01$).

Figure 4 also shows the locomotor and anxiety-like parameters of fish after acute nicotine exposure. For total distance traveled, two-way ANOVA showed a significant effect of nicotine ($F_{2,49} = 11.81$, $p < .0001$) but not of profile ($F_{1,49} = 0.12$, $p = .72$) and the interaction term profile × nicotine ($F_{2,49} = 0.02$, $p = .97$). The post hoc test indicated that bold 0.00 differed from bold 1.00, and shy 0.00 differed

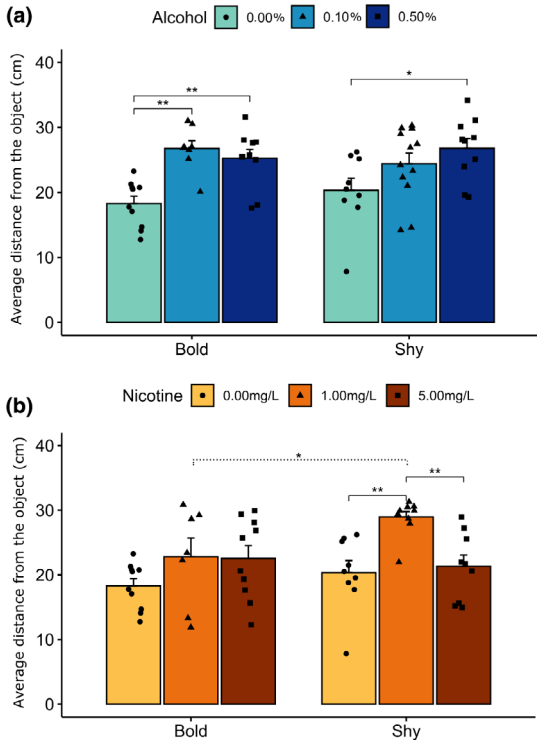

FIGURE 3 Average distance from the object (mean + SEM). Zebrafish were acutely exposed to (a) alcohol (60 min) or (b) nicotine (3 min) and then tested in the ramp tank (10 min). Solid lines indicate statistically significant differences between treatments in the same profile. Dotted lines indicate statistically significant differences among profiles (bold and shy groups). Asterisks indicate values of statistical significance (*$p \leq .05$, **$p \leq .01$).

from shy 1.00 ($p < .01$; Figure 4d). For the anxiety-like behaviors, two-way ANOVA showed that there was no statistical significance for behavioral profiles ($F_{1,49} = 2.07$, $p = .15$) and the interaction profile × nicotine ($F_{2,49} = 1.03$, $p = .36$) regarding the distance from the bottom of the tank (Figure 4e). However, statistical significance was observed for nicotine concentrations ($F_{2,49} = 13.78$, $p = .0001$), with bold 0.00 differing from bold 5.00 and shy 0.00 differing from shy 1.00 and 5.00. For time immobile (Figure 4f), two-way ANOVA revealed no statistical significance of profiles ($F_{1,49} = 1.82$, $p = .18$) and of the interaction profile × nicotine ($F_{2,49} = 0.05$, $p = .94$), but statistical significance was observed for nicotine treatment ($F_{2,49} = 31.65$, $p = .0001$). The Bonferroni test revealed that bold 0.00 showed less time immobile than bold 1.00 and 5.00, and shy 0.00 differed from shy 1.00 and 5.00 ($p < .01$; Figure 4f).

3.2 | Molecular responses

The molecular investigation revealed different gene expression patterns between different zebrafish behavioral profiles after exposure to alcohol and nicotine. The dot plot presented in Figure 5a shows an increase in the expression of genes associated directly or indirectly with alcohol. Two-way ANOVA showed statistically significant differences for some genes between alcohol treatments. For *tph1*, statistical significance was observed for alcohol treatment ($F_{2,30} = 14.6$, $p < .0001$), but no statistical significance was found for behavioral profiles ($F_{1,30} = 0.25$, $p = .61$) or the interaction profile ×

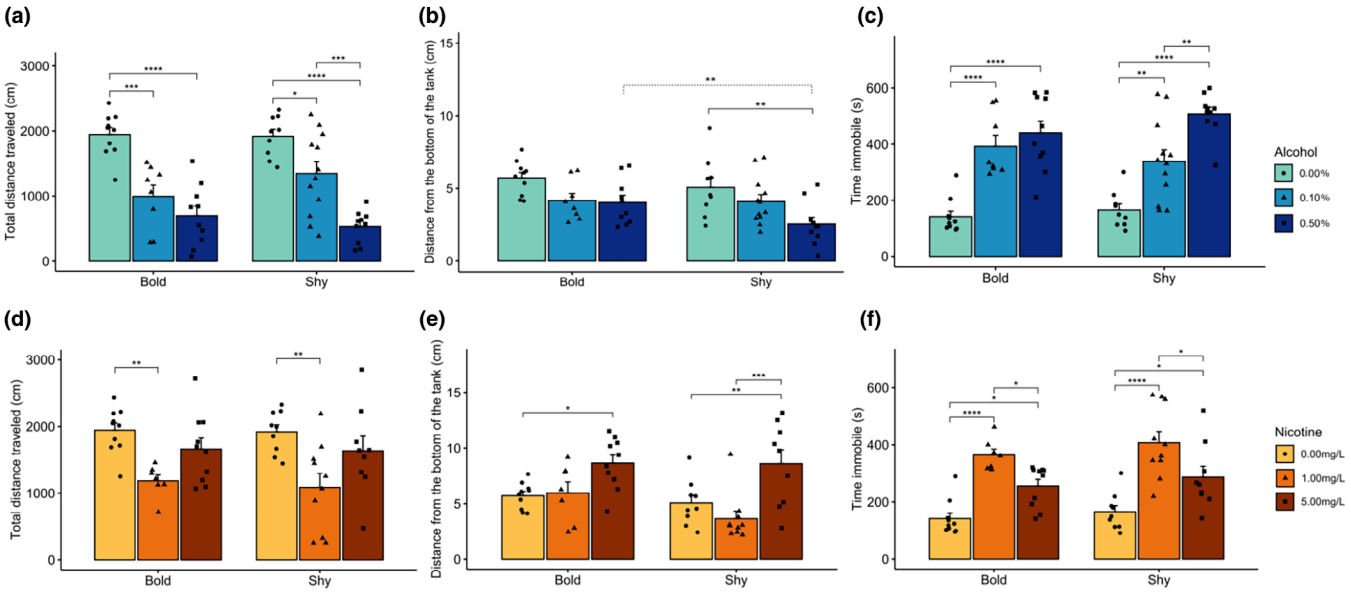

FIGURE 4 Locomotor and anxiety-like behavior of zebrafish with different profiles for 10 min in the test tank after alcohol or nicotine exposure. Results are presented as mean + SEM. (a–c) Total distance traveled (a), distance from the bottom of the tank (b), and time immobile (c) of animals treated with alcohol. (d–f) Total distance traveled (d), distance from the bottom of the tank (e), and time immobile (f) of animals treated with nicotine. Two-way ANOVA was performed. Solid lines indicate statistically significant differences between treatments for each profile. Dotted lines indicate statistically significant differences among behavioral profiles. Asterisks indicate values of statistical significance (*$p \leq .05$, **$p \leq .01$, ***$p \leq .001$, ****$p \leq .0001$).

alcohol ($F_{2,30} = 2.42$, $p = .10$). The *tph1* gene was upregulated 2.83- and 3.11-fold in bold 0.10% and 0.50% compared to bold 0.00% (Bonferroni $p < .05$).

For *gaba1*, two-way ANOVA showed statistical significance for alcohol treatment ($F_{2,30} = 6.10$, $p = .006$), but no statistical significance was found for behavioral profiles ($F_{1,30} = 0.001$, $p = .97$) or the interaction profile × alcohol ($F_{2,30} = 1.14$, $p = .33$). The *gaba1* gene was downregulated in shy 0.10% (0.87-fold), while it was upregulated 1.85-fold in shy 0.50% ($p < .05$, Bonferroni post hoc test).

Regarding *bdnf*, two-way ANOVA showed statistical significance for alcohol treatment ($F_{2,30} = 12.07$, $p = .0001$), and no statistical significance for behavioral profiles ($F_{1,30} = 2.96$, $p = .95$) or the interaction profile × alcohol ($F_{2,30} = 1.38$, $p = .26$). The *bdnf* gene was upregulated 1.58- and 1.92-fold in bold 0.50% and shy 0.50%, respectively ($p < .05$, Bonferroni post hoc test). Moreover, *bdnf* was 1.54- and 1.58-fold upregulated in bold 0.10% and 0.50% compared to bold 0.00%, respectively, and it was 1.92-fold upregulated in shy 0.50% compared to shy 0.00% ($p < .05$, post hoc test).

Concerning the mRNA levels of the other genes, there was no significant effect of profile, alcohol concentration, or profile × alcohol (*ache*—profile: $F_{1,30} = 3.15$; alcohol: $F_{2,30} = 1.35$; profile × alcohol: $F_{2,30} = 0.00$; all $p > .08$; *th1*—profile: $F_{1,30} = 0.47$; alcohol: $F_{2,30} = 2.14$; profile × alcohol: $F_{2,30} = 0.07$; all $p > .13$; *gad1b*—profile: $F_{1,30} = 0.57$; alcohol: $F_{2,30} = 0.82$; profile × alcohol: $F_{2,30} = 1.91$; for all $p > .16$).

The dot plot presented in Figure 5b shows variation in genes expression associated with nicotine exposure for bold and shy zebrafish. For *ache*, two-way ANOVA revealed statistical significance for nicotine treatment ($F_{2,30} = 10.69$, $p = .0003$) and the interaction profile × nicotine ($F_{2,30} = 3.47$, $p = .04$) between bold 1.00 (1.21-fold change) and shy 1.00 (2.33-fold change). No statistical significance was found for behavioral profiles ($F_{1,30} = 1.88$, $p = .18$). In the bold 5.00 group, *ache* was upregulated compared to bold 0.00 (4.63-fold change) and 1.00 (Bonferroni post hoc test).

For *th1*, two-way ANOVA revealed a significant effect of nicotine treatment ($F_{2,30} = 15.08$, $p < .0001$) as well as behavioral profiles ($F_{1,30} = 4.98$, $p = .03$). The Bonferroni post hoc test demonstrated downregulation in response to a concentration of 1.00 mg/L for the bold behavioral profile (0.54-fold change) and the shy behavioral profile (0.48-fold change). Furthermore, in the bold 1.00 and 5.00 groups, *th1* was downregulated (0.48- and 0.53-fold change) compared with bold 0.00 ($p < .01$, Bonferroni post hoc test). The interaction profile × nicotine was not statistically significant ($F_{2,30} = 0.69$, $p = .5$).

As regards *tph1* mRNA levels, two-way ANOVA demonstrated statistical significance for nicotine treatment ($F_{2,30} = 35.19$, $p < .0001$) and the interaction profile × nicotine ($F_{2,30} = 4.21$, $p = .02$). Compared to bold 0.00, the *tph1* gene was upregulated 3.28-fold in the bold 1.00 group ($p < .01$, Bonferroni post hoc test) and 6.34-fold in the bold 5.00 group ($p < .01$, Bonferroni post hoc test). Similarly, for the shy profile, *tph1* was upregulated 2.56-fold in the shy 1.00 group ($p < .01$, Bonferroni post hoc test) and 2.40-fold in the shy 5.00 group ($p < .01$, Bonferroni post hoc test). No significant effect was observed for behavioral profiles ($F_{1,30} = 0.33$, $p = .5$).

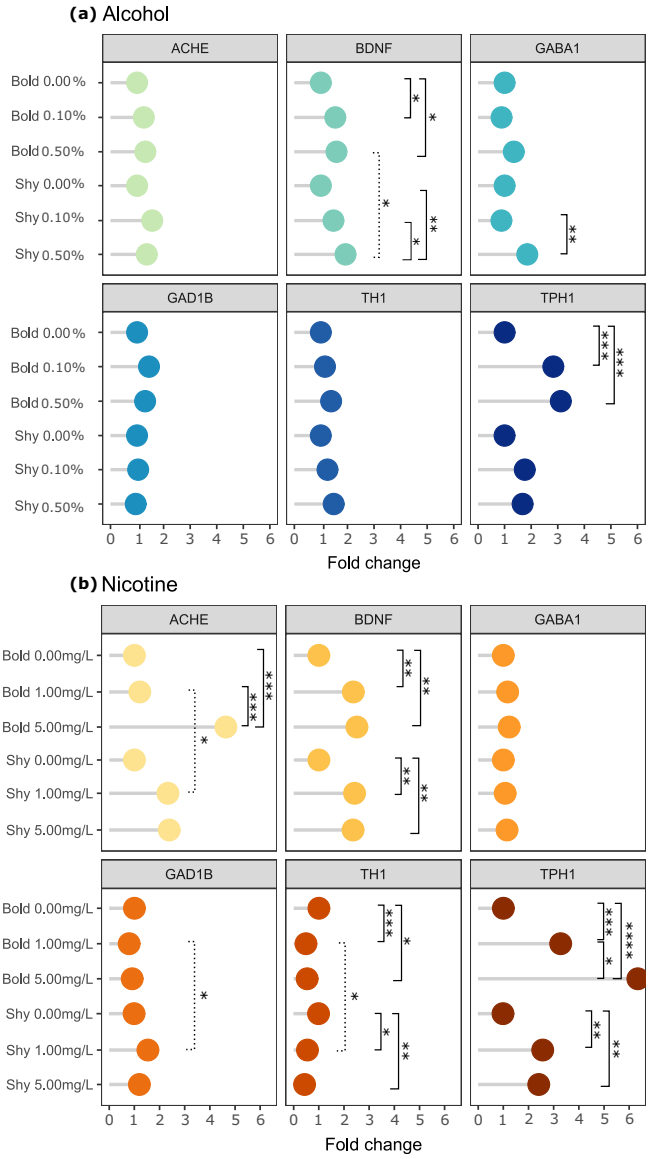

FIGURE 5 Dot plot visualization of RT-qPCR analysis of relative mRNA expression (fold change) of bold and shy zebrafish exposed to alcohol or nicotine. Target genes are known to be affected directly or indirectly by alcohol and nicotine. Acetylcholinesterase (*ache*), brain-derived neurotrophic factor (*bdnf*), $GABA_A$ receptor (*gaba1*), glutamate decarboxylase (*gad1b*), tyrosine hydroxylase (*th1*), and tryptophan hydroxylase (*tph1*) were analyzed. mRNA expression was compared between alcohol or nicotine treatments within the behavioral profiles (bold and shy). All RT-qPCR analyses were conducted in triplicate. Two-way ANOVA followed by the Bonferroni test was performed ($p < .05$). Solid lines indicate statistically significant differences between treatments in each profile. Dotted lines indicate statistically significant difference among behavioral profiles. Asterisks indicate values of statistical significance (*$p \leq .05$, **$p \leq .01$, ***$p \leq .001$, ****$p \leq .0001$).

As regards *gad1b*, two-way ANOVA demonstrated statistical significance for the interaction profile × nicotine ($F_{2,30} = 3.89$, $p = .03$), but no effect of the isolated terms nicotine treatment ($F_{2,30} = 1.14$, $p = .33$) and behavioral profiles ($F_{1,30} = 0.36$, $p = .54$). In the

bold 1.00 group, *gad1b* was downregulated (0.79-fold change) compared with the bold 0.00 group (Bonferroni post hoc test), and in the shy 1.00 group, *gad1b* was upregulated (1.53-fold) compared with the shy 0.00 group (Bonferroni post hoc test).

With respect to *bdnf* mRNA levels, two-way ANOVA demonstrated statistical significance for nicotine treatment ($F_{2,30} = 16.33$, $p < .0001$) and behavioral profiles ($F_{1,30} = 10.10$, $p = .03$), but not for the interaction profile × nicotine ($F_{2,30} = 0.00$, $p = .9$). The *bdnf* gene was 2.37- and 2.51-fold upregulated in the bold 1.00 and 5.00 groups compared with bold 0.00 ($p < .01$, Bonferroni post hoc test), and in the shy 1.00 and 5.00 groups, *bdnf* was upregulated 2.42- and 2.36-fold compared with shy 0.00 ($p < .01$, Bonferroni post hoc test). For *gaba1*, there was no significant effect of profile, nicotine treatments, or profile × nicotine (profile: $F_{1,30} = 2.90$; nicotine: $F_{2,30} = 0.59$; profile × alcohol: $F_{2,30} = 1$).

4 | DISCUSSION

In this study, we observed that alcohol and nicotine differently affect bold and shy zebrafish, both altering the behavioral response and gene expression. We applied an anxiety evaluation test from Walsh-Monteiro et al. (2016) adapted to a single ramp, which has proved to be an effective tool to highlight the differences in behavioral profiles under the effect of alcohol and nicotine. An initial dark area triggered the zebrafish's innate preference for dark hidden places (Maximino et al., 2011; Tudorache et al., 2013), whereas both the drugs (nicotine and alcohol) and fish profiles (bold and shy) led to changes in anxiety and locomotion and boosted the expression of genes related to neuronal protection (*bdnf*), toxicity (*ache*), and serotonin production (*tph1*) in the brain. This is the first study to combine gene expression and individual behavioral profiles in the analysis of the response to alcohol and nicotine. Here we found a strong relationship between serotonin synthesis (expression of *tph1*) and profiles under acute drug exposure, suggesting the serotonergic pathway plays a role in the mechanisms by which genetic risk factors affect drug use.

In the behavioral tests, in which fish explored a tank with a ramp, we observed differences in the time each animal spent in the three established areas. Control animals (bold and shy without drugs) spent more time in the ramp area independent of the behavioral profile. These findings indicate the context used triggered similar responses in bold and shy zebrafish. The tank used here not only included a ramp, which demands risk taking for exploration, but also presented a novel object. Thus, it may have imposed a high level of anxiogenic stimulus and hindered the observation of differences between bold and shy zebrafish shown in other studies (Alfonso et al., 2020; Bellot et al., 2022). In other behavioral tests, bold zebrafish were shown to present faster habituation (Found, 2019), a weaker anxiety-like response (Araujo-Silva et al., 2020), and less social attachment (Araujo-Silva et al., 2018).

However, the drug exposure showed a few differences between profiles. Alcohol exposure (0.10%) increased anxiety-like behavior only in bold zebrafish, while shy animals did not change behavior. On the other hand, 0.50% alcohol exposure caused anxiogenic effects both in bold and shy zebrafish. The reduction in locomotion and the increase in freezing observed in these animals may be explained by the alcohol and context effects, which seem to have a synergic response. Thus, our results suggest that individual differences in behavior are dependent on the interaction between the profile (bold or shy), the alcohol concentration, and the context to which the animals are exposed. These findings agree with the literature (Bellot et al., 2022; Dean et al., 2020) indicating the disparity between profiles in terms of drug responsiveness. However, we observed here that shy animals seem to be more resistant to 0.10% than bold ones, contradicting our previous studies (Araujo-Silva et al., 2018; Araujo-Silva et al., 2020). This difference may be attributed to the context used, as we have previously tested a social context, in which shy animals show high affinity. For instance, Mathur and Guo (2011) showed that shy fish exposed to 0.10% and 0.50% alcohol exhibited less tank bottom dwelling while high doses increase anxiety-like behaviors in the novel tank (a tank with no other stimuli), different from the tank used herein (novel, with ramp and object). Thus, behavioral differences in the response to alcohol seem to be context- and profile-dependent and should be considered to improve our understanding of individual differences in the response to drugs and their respective treatment designs.

Targets to treat drug use/abuse are usually the reward system, which is dominated by dopamine; however, other neurotransmitter systems are also involved in the reinforcing alcohol effects and deserve attention. According to previous research (Rico et al., 2011; Schneider, 2017), alcohol exposure modulates locomotion and anxiety by altering cholinergic, dopaminergic, serotoninergic, and GABAergic transmission, which were assessed here by analyzing the expression of *ache*, *th1*, *tph1*, *gad1b*, and *gaba1* in bold and shy zebrafish. *Ache* showed no changes upon alcohol exposure, corroborating other studies of acute exposure (Agues-Barbosa et al., 2022; Rico et al., 2007; Torres et al., 2021). Another study previously pointed out that acute alcohol exposure increases serotonin levels in zebrafish (Gerlai et al., 2009), but differences between profiles were not considered. The results obtained here indicate that bold individuals show a different pattern of brain activity under alcohol effects, which should be considered when evaluating the biological pathways underlying alcohol use risk between individual profiles. In fact, Underwood et al. (2007) showed that alcohol-addicted rats had increased levels of the tryptophan hydroxylase enzyme in the dorsal raphe region, suggesting an attempt to generate a compensatory mechanism to normalize serotonin levels. Müller, Ziani, et al. (2020) observed that *tph1* plays a role in the anxiety responses, observing a direct involvement of the serotonergic system following exposure to 0.25% and 0.50% alcohol. The behavioral effects of alcohol have been related to the agonistic action of the drug not only on the serotonergic systems, as we observed herein, but also on the GABAergic system. *Gad1b* encodes the enzyme responsible for GABA synthesis via glutamate decarboxylation. As such, the higher the expression of *gad1b*, the higher the availability of GABA in the brain (O'Connor et al., 2019). We observed

no increase in the expression of *gad1b*, which may be related to the time of exposure to the drug.

Although the neurotransmitter systems discussed here are involved in the disturbing effects of alcohol on brain function, *bdnf* expression is beneficial. *Bdnf* encodes a neurotrophin that is involved in neuroprotection, neuronal growth and differentiation, and synaptic plasticity (Lucini et al., 2018). Its increased expression in bold and shy zebrafish suggests that acute threats to brain function trigger an immediate action to protect neurons, regardless of the animal's behavioral profile. Other studies observed higher levels of *bdnf* in zebrafish exposed to drugs (Capiotti et al., 2011; Müller, Fontana, et al., 2020), in accordance with the increased expression observed here.

In contrast to the depressant effects of alcohol, nicotine is considered a stimulant drug. Both alcohol and nicotine are highly addictive. For nicotine, the behavioral tests in this study also revealed differences between behavioral profiles. We observed that shy individuals presented stronger anxiety-like responses when exposed to 1.00 mg/L nicotine compared to bold individuals, but a higher concentration of the drug induced anxiogenic responses in both profiles.

Evidence shows that acute nicotine treatment in zebrafish positively modulates cognitive responses and triggers anxiolytic responses in various behavioral tasks (Eddins et al., 2009; Levin et al., 2007; Singer et al., 2016; Ziani et al., 2018). In the present study, nicotine exposure affected the locomotor parameters by reducing the swimming speed and distance traveled in both profiles. This reduction in locomotor parameters may be related to the anxiolytic effect of nicotine, since it is mainly associated with the action on nAChRs (Bencan & Levin, 2008). These findings agree with previous studies, in which nicotine led to altered swimming patterns. We could see that bold 5.00 zebrafish were more anxious, while shy 1.00 and bold 1.00 behaved similarly.

Concerning alterations to gene expression upon acute nicotine exposure, our study reports the implication of the cholinergic system in modulating the rewarding properties of the drug. The nAChRs mediate rapid synaptic communication in neuronal synapses, playing a central role in nicotine addiction (Zarkadas et al., 2022). Another important point to highlight is that bold 1.00 zebrafish expressed less *ache* compared to shy 1.00. Moreover, cholinergic transmission modulates both the dopaminergic and serotoninergic systems (Cachope et al., 2012; Kosillo et al., 2016; Peters et al., 2021; Threlfell et al., 2012). Here, *th1* and *tph1* were down- and upregulated, respectively. The reduction in *th1* expression is expected due to the heterogeneity of nAChR subtypes, which are differently regulated by nicotine (Di Chiara, 2000; Mugnaini et al., 2006). Regarding *tph1* expression, bold individuals exposed to 5.00 mg/L nicotine showed higher *tph1* expression levels than individuals in the other groups. Considering serotonin is related to pleasure, welfare, and cognitive functions, these changes could be involved in nicotine dependence in bold animals (Herculano & Maximino, 2014; Leite-Ferreira et al., 2019).

Again, although nicotine causes several changes to brain neurotransmission, in both profiles it increased the expression of *bdnf*, which is often positively correlated with neuronal protection, synaptogenesis, and plasticity (Park & Poo, 2013). Overall, our experimental findings support the involvement of *bdnf* in nicotine-induced neurochemical changes observed in dopaminergic and serotoninergic neurons. We also analyzed the expression of *gad1b* and *gaba1* due to the potential changes in GABAergic neuronal activity in the brain after acute treatment with nicotine. No changes were observed in the expression of the *gaba1* receptor, but *gad1b* expression was higher in shy 1.00 than in bold 1.00, indicating that these profiles possibly differ in their responsiveness to these drugs.

In summary, further research is needed to explore the impact of different contextual apparatuses on zebrafish behavior and understand how specific stimuli affect their exploratory patterns. Our proposed tank presents a new approach to assessing anxiety-like behavior in zebrafish, but additional tests are necessary to validate and strengthen this method. Although the modified plus maze with a ramp offers benefits, such as evaluating predator exposure behavior, it also introduces potential confounding factors. For instance, the presence of an object on the ramp could induce anxiety, potentially influencing observed behaviors and complicating the interpretation of the results. As an alternative for future work, we suggest removing the object from the top of the ramp.

Here we observed that bold and shy individuals present different behavioral and brain activity following acute drug exposure: bold individuals showed higher sensitivity to alcohol while shy individuals were more susceptible to nicotine. Other studies are still needed to evaluate how zebrafish with different behavioral profiles deal with chronic exposure to drugs and to observe other alterations that vary according to the genetic background of the bold–shy continuum, which may indicate whether tolerance manifests differently in bold and shy individuals. Understanding the differences between the profiles of a population is essential to elucidate why individuals exhibit drug-seeking behavior and what are the consequences of long-term abuse to facilitate prevention and treatment strategies.

5 | CONCLUSION

In conclusion, we examined some changes in behavior and gene expression resulting from acute alcohol and nicotine exposure in zebrafish with different behavioral profiles. The results obtained are robust and confirm that there are differences between bold and shy zebrafish from the behavioral and genetic points of view, which were shown to be context-, drug concentration-, and profile-dependent. To better elucidate the effects of alcohol and nicotine on the neurophysiology of individuals and suggest new addiction treatments, new studies correlating dopamine and serotonin levels are needed to close the gaps that permeate studies involving behavioral profiles and drug effects on the nervous system.

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REFERENCES

Agues-Barbosa, T., da Silva Junior, F. C., Gomes-de-Lima, J. N., Batistuzzo de Medeiros, S. R., & Luchiari, A. C. (2022). Behavioral genetics of alcohol's effects in three zebrafish (Danio rerio) populations. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *114*(2021), 110495. https://doi.org/10.1016/j.pnpbp.2021.110495

Alfonso, S., Peyrafort, M., Cousin, X., & Bégout, M. L. (2020). Zebrafish Danio rerio shows behavioural cross-context consistency at larval and juvenile stages but no consistency between stages. *Journal of Fish Biology*, *96*(6), 1411–1421. https://doi.org/10.1111/jfb.14310

Alfonso, S., Sadoul, B., Gesto, M., Joassard, L., Chatain, B., Geffroy, B., & Bégout, M.-L. (2019). Coping styles in European sea bass: The link between boldness, stress response and neurogenesis. *Physiology & Behavior*, *207*(2018), 76–85. https://doi.org/10.1016/j.physbeh.2019.04.020

Araujo-Silva, H., Leite-Ferreira, M. E., & Luchiari, A. C. (2020). Behavioral screening of alcohol effects and individual differences in zebrafish (Danio rerio). *Alcohol and Alcoholism*, *55*(6), 1–7. https://doi.org/10.1093/alcalc/agaa046

Araujo-Silva, H., Pinheiro-da-Silva, J., Silva, P. F., & Luchiari, A. C. (2018). Individual differences in response to alcohol exposure in zebrafish (Danio rerio). *PLoS One*, *13*(6), 1–12. https://doi.org/10.1371/journal.pone.0198856

Bahi, A. (2013). Increased anxiety, voluntary alcohol consumption and ethanol-induced place preference in mice following chronic psychosocial stress. *Stress*, *16*(4), 441–451. https://doi.org/10.3109/10253890.2012.754419

Banerjee, N. (2014). Neurotransmitters in alcoholism: A review of neurobiological and genetic studies. *Indian Journal of Human Genetics*, *20*(1), 20–31. https://doi.org/10.4103/0971-6866.132750

Bellot, M. S., Guermandi, I. I., Camargo-dos-Santos, B., & Giaquinto, P. C. (2022). Differences in the alcohol preference assessment of shy and bold zebrafish. *Frontiers in Behavioral Neuroscience*, *16*(2), 1–8. https://doi.org/10.3389/fnbeh.2022.810051

Bencan, Z., & Levin, E. D. (2008). The role of α7 and α4β2 nicotinic receptors in the nicotine-induced anxiolytic effect in zebrafish. *Physiology & Behavior*, *95*(3), 408–412. https://doi.org/10.1016/j.physbeh.2008.07.009

Benowitz, N. L. (2010). Nicotine addiction. *New England Journal of Medicine*, *362*(24), 2295–2303. https://doi.org/10.1056/NEJMra0809890

Bobo, J. K., & Husten, C. (2000). Sociocultural influences on smoking and drinking. *Alcohol Research & Health: The Journal of the National Institute on Alcohol Abuse and Alcoholism*, *24*(4), 225–232. Retrieved from: http://www.ncbi.nlm.nih.gov/pubmed/15986717

Breslau, N. (1995). Psychiatric comorbidity of smoking and nicotine dependence. *Behavior Genetics*, *46*(8), 867–876. https://doi.org/10.1177/1403494817746534

Cachope, R., Mateo, Y., Mathur, B. N., Irving, J., Wang, H.-L., Morales, M., Lovinger, D. M., & Cheer, J. F. (2012). Selective activation of cholinergic interneurons enhances Accumbal phasic dopamine release: Setting the tone for reward processing. *Cell Reports*, *2*(1), 33–41. https://doi.org/10.1016/j.celrep.2012.05.011

Capiotti, K. M., Menezes, F. P., Nazario, L. R., Pohlmann, J. B., de Oliveira, G. M. T., Fazenda, L., Bogo, M. R., Bonan, C. D., & da Silva, R. S. (2011). Early exposure to caffeine affects gene expression of adenosine receptors, DARPP-32 and BDNF without affecting sensibility and morphology of developing zebrafish (Danio rerio). *Neurotoxicology and Teratology*, *33*(6), 680–685. https://doi.org/10.1016/j.ntt.2011.08.010

Carere, C., Drent, P. J., Privitera, L.., Koolhaas, J. M., & Groothuis, T. G. (2005). Personalities in great tits, Parus major: Stability and consistency. *Animal Behaviour*, *70*(4), 795–805.

Cavigelli, S. A. (2005). Animal personality and health. *Behaviour*, *142*(9), 1223–1244. https://doi.org/10.1163/156853905774539355

Cui, W.-Y., Seneviratne, C., Gu, J., & Li, M. D. (2012). Genetics of GABAergic signaling in nicotine and alcohol dependence. *Human Genetics*, *131*(6), 843–855. https://doi.org/10.1007/s00439-011-1108-4

Daniel, D. K., & Bhat, A. (2020). Bolder and brighter? Exploring correlations between personality and cognitive abilities among individuals within a population of wild zebrafish, Danio Rerio. *Frontiers in Behavioral Neuroscience*, *14*, 1–13. https://doi.org/10.3389/fnbeh.2020.00138

Davies, M. (2003). The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. *Journal of Psychiatry and Neuroscience*, *28*(4), 263–274.

Dean, R., Duperreault, E., Newton, D., Krook, J., Ingraham, E., Gallup, J., Franczak, B. C., & Hamilton, T. J. (2020). Opposing effects of acute and repeated nicotine exposure on boldness in zebrafish. *Scientific Reports*, *10*(1), 8570. https://doi.org/10.1038/s41598-020-65382-6

Di Chiara, G. (2000). Role of dopamine in the behavioural actions of nicotine related to addiction. *European Journal of Pharmacology*, *393*(1–3), 295–314. https://doi.org/10.1016/S0014-2999(00)00122-9

Echevarria, D. J., Toms, C. N., & Jouandot, D. J. (2011). Alcohol-induced behavior change in zebrafish models. *Reviews in the Neurosciences*, *22*(1), 85–93. https://doi.org/10.1515/RNS.2011.010

Eddins, D., Petro, A., Williams, P., Cerutti, D. T., & Levin, E. D. (2009). Nicotine effects on learning in zebrafish: The role of dopaminergic systems. *Psychopharmacology*, *202*(1–3), 103–109. https://doi.org/10.1007/s00213-008-1287-4

Elvig, S. K., McGinn, M. A., Smith, C., Arends, M. A., Koob, G. F., & Vendruscolo, L. F. (2021). Tolerance to alcohol: A critical yet understudied factor in alcohol addiction. *Pharmacology Biochemistry and Behavior*, *204*(October 2020), 173155. https://doi.org/10.1016/j.pbb.2021.173155

Ferrari, S., Millot, S., Leguay, D., Chatain, B., & Bégout, M.-L. (2015). Consistency in European seabass coping styles: A life-history approach. *Applied Animal Behaviour Science*, *167*, 74–88. https://doi.org/10.1016/j.applanim.2015.03.006

Found, R. (2019). Personality influences habituation behaviour in ungulates. *Journal of Ethology*, *37*(1), 47–58. https://doi.org/10.1007/s10164-018-0567-7

Gerlai, R., Chatterjee, D., Pereira, T., Sawashima, T., & Krishnannair, R. (2009). Acute and chronic alcohol dose: Population differences in behavior and neurochemistry of zebrafish. *Genes, Brain and Behavior*, *8*(6), 586–599. https://doi.org/10.1111/j.1601-183X.2009.00488.x

Goldman, D., Oroszi, G., & Ducci, F. (2005). The genetics of addictions: Uncovering the genes. *Nature Reviews Genetics*, *6*(7), 521–532. https://doi.org/10.1038/nrg1635

Gosling, S. D., & John, O. P. (1999). Personality dimensions in nonhuman animals: A cross-species review. *Current Directions in Psychological Science*, *8*(3), 69–75.

Hendrickson, L. M., Guildford, M. J., & Tapper, A. R. (2013). Neuronal nicotinic acetylcholine receptors: Common molecular substrates of nicotine and alcohol dependence. *Frontiers in Psychiatry*, *4*, 29. https://doi.org/10.3389/fpsyt.2013.00029

Herculano, A. M., & Maximino, C. (2014). Serotonergic modulation of zebrafish behavior: Towards a paradox. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *55*, 50–66. https://doi.org/10.1016/j.pnpbp.2014.03.008

Huntingford, F. A., Andrew, G., Mackenzie, S., Morera, D., Coyle, S. M., Pilarczyk, M., & Kadri, S. (2010). Coping strategies in a strongly schooling fish, the common carp Cyprinus carpio. *Journal of Fish Biology*, *76*(7), 1576–1591. https://doi.org/10.1111/j.1095-8649.2010.02582.x

Johnson, B. A. (2004). Role of the serotonergic system in the neurobiology of alcoholism: Implications for treatment. *CNS Drugs*, *18*(15), 1105–1118. https://doi.org/10.2165/00023210-200418150-00005

Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in Pharmacological Sciences*, *35*(2), 63–75. https://doi.org/10.1016/j.tips.2013.12.002

Kanai, R., & Rees, G. (2011). The structural basis of inter-individual differences in human behaviour and cognition. *Nature Reviews Neuroscience*, *12*(4), 231–242. https://doi.org/10.1038/nrn3000

Kassambara, A. (2020a). Package 'ggpubr.'. Retrieved from R package website: https://rpkgs.datanovia.com/ggpubr/.

Kassambara, A. (2020b). rstatix: pipe-friendly framework for basic statistical tests. R package version 0.4. 0. Retrieved from R package website: https://rpkgs.datanovia.com/rstatix/.

Klee, E. W., Ebbert, J. O., Schneider, H., Hurt, R. D., & Ekker, S. C. (2011). Zebrafish for the study of the biological effects of nicotine. *Nicotine & Tobacco Research*, *13*(5), 301–312. https://doi.org/10.1093/ntr/ntr010

Klee, E. W., Schneider, H., Clark, K. J., Cousin, M. A., Ebbert, J. O., Hooten, W. M., Karpyak, V. M., Warner, D. O., & Ekker, S. C. (2012). Zebrafish: A model for the study of addiction genetics. *Human Genetics*, *131*(6), 977–1008. https://doi.org/10.1007/s00439-011-1128-0

Koob, G. F. (2014). Neurocircuitry of alcohol addiction: Synthesis from animal models. In *Handbook of clinical neurology* (Vol. 125, 1st ed., pp. 33–54). Elsevier. https://doi.org/10.1016/B978-0-444-62619-6.00003-3

Koob, G. F., & Colrain, I. M. (2020). Alcohol use disorder and sleep disturbances: A feed-forward allostatic framework. *Neuropsychopharmacology*, *45*(1), 141–165. https://doi.org/10.1038/s41386-019-0446-0

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, *35*(1), 217–238. https://doi.org/10.1038/npp.2009.110

Koolhaas, J. M., Korte, S. M., de Boer, S. F., van der Vegt, B. J., van Reenen, C. G., Hopster, H., de Jong, I. C., Ruis, M. A. W., & Blokhuis, H. J. (1999). Coping styles in animals: Current status in behavior and stress physiology. *Neuroscience and Biobehavioral Reviews.*, *23*, 925–935. https://doi.org/10.1016/S0149-7634(99)00026-3

Kosillo, P., Zhang, Y.-F., Threlfell, S., & Cragg, S. J. (2016). Cortical control of striatal dopamine transmission via striatal cholinergic interneurons. *Cerebral Cortex*, *26*(11), 4160–4169. https://doi.org/10.1093/cercor/bhw252

Leite-Ferreira, M. E., Araujo-Silva, H., & Luchiari, A. C. (2019). Individual differences in hatching time predict alcohol response in zebrafish. *Frontiers in Behavioral Neuroscience*, *13*(7), 1–9. https://doi.org/10.3389/fnbeh.2019.00166

Lenth, R., & Lenth, M. R. (2018). Package 'lsmeans'. *The American Statistician*, *34*(4), 216–221.

Levin, E. D., Bencan, Z., & Cerutti, D. T. (2007). Anxiolytic effects of nicotine in zebrafish. *Physiology & Behavior*, *90*(1), 54–58. https://doi.org/10.1016/j.physbeh.2006.08.026

Lucini, C., D'angelo, L., Cacialli, P., Palladino, A., & de Girolamo, P. (2018). BDNF, brain, and regeneration: Insights from zebrafish. *International Journal of Molecular Sciences*, *19*(10), 1–16. https://doi.org/10.3390/ijms19103155

Mamuneas, D., Spence, A. J., Manica, A., & King, A. J. (2015). Bolder stickleback fish make faster decisions, but they are not less accurate. *Behavioral Ecology*, *26*(1), 91–96. https://doi.org/10.1093/beheco/aru160

Mathur, P., & Guo, S. (2010). Use of zebrafish as a model to understand mechanisms of addiction and complex neurobehavioral phenotypes. *Neurobiology of Disease*, *40*(1), 66–72. https://doi.org/10.1016/j.nbd.2010.05.016

Mathur, P., & Guo, S. (2011). Differences of acute versus chronic ethanol exposure on anxiety-like behavioral responses in zebrafish. *Behavioural Brain Research*, *219*(2), 234–239. https://doi.org/10.1016/j.bbr.2011.01.019

Maximino, C., da Silva, A. W. B., Gouveia, A., & Herculano, A. M. (2011). Pharmacological analysis of zebrafish (Danio rerio) scototaxis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry.*, *35*, 624–631. https://doi.org/10.1016/j.pnpbp.2011.01.006

Miller, N., Greene, K., Dydinski, A., & Gerlai, R. (2013). Effects of nicotine and alcohol on zebrafish (Danio rerio) shoaling. *Behavioural Brain Research*, *240*(1), 192–196. https://doi.org/10.1016/j.bbr.2012.11.033

Millot, S., Bégout, M.-L., & Chatain, B. (2009). Exploration behaviour and flight response toward a stimulus in three sea bass strains (Dicentrarchus labrax L.). *Applied Animal Behaviour Science*, *119*(1–2), 108–114. https://doi.org/10.1016/j.applanim.2009.03.009

Moscicki, M. K., & Hurd, P. L. (2015). Sex, boldness and stress experience affect convict cichlid, Amatitlania nigrofasciata, open field behaviour. *Animal Behaviour*, *107*, 105–114. https://doi.org/10.1016/j.anbehav.2015.05.027

Mugnaini, M., Garzotti, M., Sartori, I., Pilla, M., Repeto, P., Heidbreder, C. A., & Tessari, M. (2006). Selective down-regulation of [125I]Y0-α-conotoxin MII binding in rat mesostriatal dopamine pathway following continuous infusion of nicotine. *Neuroscience*, *137*(2), 565–572. https://doi.org/10.1016/j.neuroscience.2005.09.008

Müller, T. E., Fontana, B. D., Bertoncello, K. T., Franscescon, F., Mezzomo, N. J., Canzian, J., Stefanello, F. V., Parker, M. O., Gerlai, R., & Rosemberg, D. B. (2020). Understanding the neurobiological effects of drug abuse: Lessons from zebrafish models. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *100*(1), 109873. https://doi.org/10.1016/j.pnpbp.2020.109873

Müller, T. E., Ziani, P. R., Fontana, B. D., Duarte, T., Stefanello, F. V., Canzian, J., Santos, A. R. S., & Rosemberg, D. B. (2020). Role of the serotonergic system in ethanol-induced aggression and anxiety: A pharmacological approach using the zebrafish model. *European Neuropsychopharmacology*, *32*, 66–76. https://doi.org/10.1016/j.euroneuro.2019.12.120

O'Connor, M. J., Beebe, L. L., Deodato, D., Ball, R. E., Page, A. T., VanLeuven, A. J., Harris, K. T., Park, S., Hariharan, V., Lauderdale, J. D., & Dore, T. M. (2019). Bypassing glutamic acid decarboxylase 1 (Gad1) induced craniofacial defects with a photoactivatable translation blocker morpholino. *ACS Chemical Neuroscience*, *10*(1), 266–278. https://doi.org/10.1021/acschemneuro.8b00231

Oswald, M. E., Drew, R. E., Racine, M., Murdoch, G. K., & Robison, B. D. (2012). Is behavioral variation along the bold-shy continuum associated with variation in the stress axis in zebrafish? *Physiological and Biochemical Zoology*, *85*(6), 717–728. https://doi.org/10.1086/668203

Øverli, Ø., Korzan, W. J., Höglund, E., Winberg, S., Bollig, H., Watt, M., Forster, G. L., Barton, B. A., Øverli, E., Renner, K. J., & Summers, C. H. (2004). Stress coping style predicts aggression and social dominance in rainbow trout. *Hormones and Behavior*, *45*(4), 235–241. https://doi.org/10.1016/j.yhbeh.2003.12.002

Øverli, Ø., Sørensen, C., Pulman, K. G. T., Pottinger, T. G., Korzan, W., Summers, C. H., & Nilsson, G. E. (2007). Evolutionary background for stress-coping styles: Relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neuroscience & Biobehavioral Reviews*, *31*(3), 396–412. https://doi.org/10.1016/j.neubiorev.2006.10.006

Park, H., & Poo, M. (2013). Neurotrophin regulation of neural circuit development and function. *Nature Reviews Neuroscience*, *14*(1), 7–23. https://doi.org/10.1038/nrn3379

Peters, K. Z., Cheer, J. F., & Tonini, R. (2021). Modulating the neuromodulators: Dopamine, serotonin, and the endocannabinoid system. *Trends in Neurosciences*, *44*(6), 464–477. https://doi.org/10.1016/j.tins.2021.02.001

Pinheiro-da-Silva, J., Silva, P. F., Nogueira, M. B., & Luchiari, A. C. (2017). Sleep deprivation effects on object discrimination task in zebrafish (Danio rerio). *Animal Cognition*, *20*(2), 159–169. https://doi.org/10.1007/s10071-016-1034-x

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*(2), 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x

Rico, E. P., Rosemberg, D. B., Dias, R. D., Bogo, M. R., & Bonan, C. D. (2007). Ethanol alters acetylcholinesterase activity and gene expression in zebrafish brain. *Toxicology Letters*, *174*(1–3), 25–30. https://doi.org/10.1016/j.toxlet.2007.08.005

Rico, E. P., Rosemberg, D. B., Seibt, K. J., Capiotti, K. M., Da Silva, R. S., & Bonan, C. D. (2011). Zebrafish neurotransmitter systems as potential pharmacological and toxicological targets. *Neurotoxicology and Teratology*, *33*(6), 608–617. https://doi.org/10.1016/j.ntt.2011.07.007

Roberto, M., Cruz, M. T., Gilpin, N. W., Sabino, V., Schweitzer, P., Bajo, M., Cottone, P., Madamba, S. G., Stouffer, D. G., Zorrilla, E. P., Koob, G. F., Siggins, G. R., & Parsons, L. H. (2010). Corticotropin releasing factor-induced amygdala gamma-aminobutyric acid release plays a key role in alcohol dependence. *Biological Psychiatry*, *67*(9), 831–839. https://doi.org/10.1016/j.biopsych.2009.11.007

Schneider, H. (2017). Zebrafish neurobehavioral assays for drug addiction research. In A. V. Kalueff (Ed.), *The rights and wrongs of zebrafish: Behavioral phenotyping of zebrafish* (pp. 171–205). Springer International Publishing. https://doi.org/10.1007/978-3-319-33774-6_8

Sherafat, Y., Bautista, M., & Fowler, C. D. (2021). Multidimensional intersection of nicotine, gene expression, and behavior. *Frontiers in Behavioral Neuroscience*, *15*(3), 1–18. https://doi.org/10.3389/fnbeh.2021.649129

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral syndromes: An integrative overview. *Quarterly Review of Biology*, *79*(3), 241–277. https://doi.org/10.1086/422893

Singer, M. L., Oreschak, K., Rhinehart, Z., & Robison, B. D. (2016). Anxiolytic effects of fluoxetine and nicotine exposure on exploratory behavior in zebrafish. *PeerJ*, *4*(8), e2352. https://doi.org/10.7717/peerj.2352

Skóra, M. N., Pattij, T., Beroun, A., Kogias, G., Mielenz, D., de Vries, T., Radwanska, K., & Müller, C. P. (2020). Personality driven alcohol and drug abuse: New mechanisms revealed. *Neuroscience & Biobehavioral Reviews*, *116*(June), 64–73. https://doi.org/10.1016/j.neubiorev.2020.06.023

Stamps, J. A. (2007). Growth-mortality tradeoffs and Personality traits in animals. *Ecology Letters*, *10*(5), 355–363. https://doi.org/10.1111/j.1461-0248.2007.01034.x

Svartberg, K. (2005). A comparison of behaviour in test and in everyday life: Evidence of three consistent boldness-related personality traits in dogs. *Applied Animal Behaviour Science*, *91*(1–2), 103–128. https://doi.org/10.1016/j.applanim.2004.08.030

Tarren, J., Shariff, M., Holgate, J., & Bartlett, S. E. (2016). Effects of alcohol on nicotinic acetylcholine receptors and impact on addiction. In *Neuropathology of drug addictions and substance misuse* (Vol. 1, pp. 411–419). Elsevier. https://doi.org/10.1016/B978-0-12-800213-1.00038-9

Threlfell, S., Lalic, T., Platt, N. J., Jennings, K. A., Deisseroth, K., & Cragg, S. J. (2012). Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. *Neuron*, *75*(1), 58–64. https://doi.org/10.1016/j.neuron.2012.04.038

Torres, C. A., Mendes, N. V., Baldin, S. L., Bernardo, H. T., Vieira, K. M., Scussel, R., de Bem Silveira, G., Silveira, P. C. L., Machado-de-Ávila, R. A., & Rico, E. P. (2021). Cotreatment of small gold nanoparticles protects against the increase in cerebral acetylcholinesterase activity and oxidative stress induced by acute ethanol exposure in the zebrafish. *Neuroscience*, *457*, 41–50. https://doi.org/10.1016/j.neuroscience.2021.01.011

Tran, S., Nowicki, M., Facciol, A., Chatterjee, D., & Gerlai, R. (2016). Ethanol-induced ADH activity in zebrafish: Differential concentration-dependent effects on high- versus low-affinity ADH enzymes 1. *Zebrafish*, *13*(2), 75–78. https://doi.org/10.1089/zeb.2015.1173

Tudorache, C., Schaaf, M. J. M., & Slabbekoorn, H. (2013). Covariation between behaviour and physiology indicators of coping style in zebrafish (Danio rerio). *Journal of Endocrinology*, *219*(3), 251–258. https://doi.org/10.1530/JOE-13-0225

Underwood, M. D., Mann, J. J., & Arango, V. (2007). Morphometry of dorsal raphe nucleus serotonergic neurons in alcoholism. *Alcoholism: Clinical and Experimental Research*, *31*(5), 837–845. https://doi.org/10.1111/j.1530-0277.2007.00365.x

Walsh-Monteiro, A., Pessoa, R., dos, S., Sanches, É. M., Carvalho, A. C. C., de Silva, Y. C., de, B. E., Chirinéa, G., & Gouveia, A. (2016). A new anxiety test for zebrafish: Plus maze with ramp. *Psychology & Neuroscience*, *9*(4), 457–464. https://doi.org/10.1037/pne0000067

Wang, M.-Y., Brennan, C. H., Lachlan, R. F., & Chittka, L. (2015). Speed–accuracy trade-offs and individually consistent decision making by individuals and dyads of zebrafish in a colour discrimination task. *Animal Behaviour*, *103*, 277–283. https://doi.org/10.1016/j.anbehav.2015.01.022

White, S. L., Wagner, T., Gowan, C., & Braithwaite, V. A. (2017). Can personality predict individual differences in brook trout spatial learning ability? *Behavioural Processes*, *141*, 220–228. https://doi.org/10.1016/j.beproc.2016.08.009

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wills, L., & Kenny, P. J. (2021). Addiction-related neuroadaptations following chronic nicotine exposure. *Journal of Neurochemistry*, *157*(5), 1652–1673. https://doi.org/10.1111/jnc.15356

Wilson, D. S., Clark, A. B., Coleman, K., & Dearstyne, T. (1994). Shyness and boldness in humans and other animals. *Trends in Ecology & Evolution*, *9*(11), 442–446. https://doi.org/10.1016/0169-5347(94)90134-1

Wingo, T., Nesil, T., Choi, J.-S., & Li, M. D. (2016). Novelty seeking and drug addiction in humans and animals: From behavior to molecules. *Journal of Neuroimmune Pharmacology*, *11*(3), 456–470. https://doi.org/10.1007/s11481-015-9636-7

Wolf, M., van Doorn, G. S., Leimar, O., & Weissing, F. J. (2007). Life-history trade-offs favour the evolution of animal personalities. *Nature*, *447*(7144), 581–584. https://doi.org/10.1038/nature05835

Xu, C., & Zon, L. I. (2010). The zebrafish as a model for human disease. In *Fish physiology* (Vol. 29, pp. 345–365). Elsevier. https://doi.org/10.1016/S1546-5098(10)02909-2

Zarkadas, E., Pebay-Peyroula, E., Thompson, M. J., Schoehn, G., Uchański, T., Steyaert, J., Chipot, C., Dehez, F., Baenziger, J. E., & Nury, H. (2022). Conformational transitions and ligand-binding to a muscle-type nicotinic acetylcholine receptor. *Neuron*, *110*(8), 1358–1370.e5. https://doi.org/10.1016/j.neuron.2022.01.013

Ziani, P. R., Müller, T. E., Stefanello, F. V., Fontana, B. D., Duarte, T., Canzian, J., & Rosemberg, D. B. (2018). Nicotine increases fear responses and brain acetylcholinesterase activity in a context-dependent manner in zebrafish. *Pharmacology Biochemistry and Behavior*, *170*(4), 36–43. https://doi.org/10.1016/j.pbb.2018.05.004

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution.*, *1*, 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

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