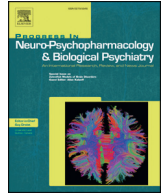




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Neurochemical factors underlying individual differences in locomotor activity and anxiety-like behavioral responses in zebrafish

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ABSTRACT

Variation among individuals may arise for several reasons, and may have diverse underlying mechanisms. Individual differences have been studied in a variety of species, but recently a new model organism has emerged in this field that offers both sophistication in phenotypical characterization and powerful mechanistic analysis. Recently, zebrafish, one of the favorites of geneticists, have been shown to exhibit consistent individual differences in baseline locomotor activity. In the current study, we further explore this finding and examine whether individual differences in locomotor activity correlate with anxiety-like behavioral measures and with levels of dopamine, serotonin and the metabolites of these neurotransmitters. In addition, we examine whether individual differences in locomotor activity are also associated with reactivity to the locomotor stimulant effects of and neurochemical responses to acute ethanol exposure (30 min long, 1% v/v ethanol bath application). Principal component analyses revealed a strong association among anxiety-like responses, locomotor activity, serotonin and dopamine levels. Furthermore, ethanol exposure was found to abolish the locomotion-dependent anxiety-like behavioral and serotonergic responses suggesting that this drug also engages a common underlying pathway. Overall, our results provide support for an important role of the serotonergic system in mediating individual differences in anxiety-like responses and locomotor activity in zebrafish and for a minor modulatory role of the dopaminergic system.

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1. Introduction

Zebrafish have been shown to exhibit inter-individual variation in behavior (Oswald et al., 2012, 2013; Toms and Echevarria, 2014; Tran and Gerlai, 2013a; Wisenden et al., 2011). The exact origin of these differences is unknown, but they could arise as a result of genetic effects, variation in the environment and/or genotype × environment interaction (Gerlai and Csányi, 1990). Irrespective of the actual origin of individual differences, the mechanisms mediating them may involve a large number of processes, including neurochemical differences among the individuals (Oswald et al., 2012, 2013; Wisenden et al., 2011). Individual differences in behavioral performance among

zebrafish have been the subject of recent attention with 5 different behavioral axes commonly examined: boldness, aggressiveness, sociability, exploration, and activity (Ariyomo and Watt, 2012; Ariyomo et al., 2013; Conrad et al., 2011; Toms and Echevarria, 2014). Differences along these behavioral features may also cluster together forming behavioral syndromes (Dahlbom et al., 2011; Moretz et al., 2007). Recently, we demonstrated that zebrafish from a genetically heterogeneous wild-type population exhibit consistent individual differences in locomotor activity quantified by total distance traveled over several behavioral testing sessions and across different behavioral paradigms (Tran and Gerlai, 2013a). Furthermore, activity levels in this latter study also correlated with the distance zebrafish swam from the bottom of the tank, a measure often utilized to quantify level of anxiety (Levin et al., 2007; Nowicki et al., 2014). The correlation between activity levels and distance to bottom may represent a behavioral syndrome warranting further investigation including different behavioral measures of anxiety.

In zebrafish, locomotor activity (e.g. total distance traveled or ambulation scores) and anxiety-like behavioral responses (e.g. distance to bottom, absolute turn angle, or variance of distance to bottom) are two of the most common behavioral parameters examined (Bencan and Levin, 2008; Bencan et al., 2009; Blaser and Gerlai, 2006; Levin et al., 2007; Nowicki et al., 2014; Tran and Gerlai, 2013b). Numerous

Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HPLC, high precision liquid chromatography; PCA, principal component analysis; ANOVA, analysis of variance; HSD, Honestly Significant Difference; D1-R, D₁ receptor; SF, short fin; VMAT, vesicular monoamine transporter.

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studies have been published with the goal of trying to identify the neurochemical and physiological mechanisms mediating these behavioral responses (Tran et al., 2015). Current pharmacological evidence suggests that the dopaminergic system mediates locomotor activity (Irons et al., 2013; Tran et al., 2014) and the serotonergic system mediates anxiety-like behavioral responses in zebrafish (Bencan et al., 2009; Connors et al., 2014; Maximino et al., 2013a). For example, exposure to a dopamine D₁ receptor antagonist has been shown to suppress locomotor activity (Irons et al., 2013; Tran et al., 2014) and to decrease the levels of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the zebrafish brain (Scerbina et al., 2012). Moreover, extracellular levels of serotonin in the brain have been correlated with changes in anxiety-like behaviors induced by a novel environment (Maximino et al., 2013a). However, it is currently unknown whether individual variation in locomotor activity and anxiety-like behavioral responses correlate with each other and/or with functioning of the dopaminergic and serotonergic neurotransmitter systems.

Individual differences in mechanisms that underlie the previously observed variation in locomotor activity of the experimental subjects may also influence how these fish respond to alcohol. In rodents, individual differences have been demonstrated in novelty induced hyper-activity (Clinton et al., 2014; Flagel et al., 2014), and high responders (HR) versus low responders (LR) have been found to exhibit robust differences when exposed to drugs of abuse including ethanol (Arias et al., 2009). For example, HR rats exhibit a significant increase in locomotor activity in response to a stimulatory dose of ethanol compared to LR rats (Gringras and Cools, 1996). It is currently unknown whether individual differences in zebrafish locomotor activity affect how they respond to stimulant doses of ethanol.

In the current study, we examine whether individual differences in locomotor activity correlate with different behavioral measures of anxiety and with levels of neurotransmitters dopamine, serotonin and their metabolites. In addition, we also examine whether behavioral and neurochemical responses to acute ethanol exposure are dependent upon individual differences in locomotor activity.

2. Materials and methods

2.1. Animals and housing

100 short-fin wild-type male and female sexually mature young adult zebrafish of the same size (about 3 cm total length) were obtained from Big Al's Aquarium (Mississauga, ON, Canada). Although the exact chronological age of the fish could not be determined, the biological age (developmental stage) of fish and their locomotory behavior correlate closely with their size (Gilbert et al., 2014) and we estimate that the fish we obtained were about 4–6 months old. This random-bred wild type population is expected to exhibit a high genetic as well as environmental variance due to the large effective population size (number of individuals used for breeding), uncontrolled breeding (reduced probability of brother sister mating) and variable environment in which they were raised and maintained.

Zebrafish were housed in 37 L Plexi glass tanks with biological and carbon filtration (n = 20 per tank) for a minimum of 2 weeks prior to behavioral testing. Zebrafish were fed twice a day with brine shrimp and a mixture of dried flake food (2:1 Aquaneering dried food and Spirulina). Animals were kept on a 14:10 h light–dark cycle with lights turning on at 08:00 and off at 22:00. Detailed information regarding animal housing and maintenance is described elsewhere (Tran and Gerlai, 2013a,b).

2.2. Experimental design and testing procedure

Since locomotor activity of zebrafish largely depends on how well fed the subjects are (Novak et al., 2005), experimental zebrafish were all treated in the same manner and were fed the night before but not

in the morning immediately prior to experimental testing. Individual zebrafish were netted from their 37 L home tanks and placed in a 1.5 L novel tank (height × width × top × bottom, 15 × 28 × 22.5 × 7 cm) filled with system water for 10 min to determine baseline locomotor activity as quantified by total distance traveled. The total distance traveled during the entire 10 min of observation was quantified for each fish. Zebrafish were subsequently ranked from highest to lowest based on the total distance traveled and three separate activity groups “high”, “medium”, and “low” were created based on percentile rankings of locomotor activity scores as described previously (Tran and Gerlai, 2013a) with 33–34 per group. To examine the effect of individual differences in ethanol reactivity, individual zebrafish in each activity group were subsequently exposed to either 0 or 1% v/v ethanol in a 1.5 L tank identical in appearance and dimensions to the initial novel tank (height × width × top × bottom, 15 × 28 × 22.5 × 7 cm) for 30 min (16–17 per group). Since the drug exposure tank was identical to the initial novel tank, the baseline quantification also served as a habituation session for zebrafish. The duration and concentration of ethanol used are based on the known concentration- and time-dependent locomotor stimulant effects of this substance published previously (Tran and Gerlai, 2013b). In this latter study, we reported that exposure to 1% ethanol increases locomotor activity with a peak observed at approximately 20 to 30 min post-exposure with brain alcohol levels approaching equilibrium by the end of this period (Tran and Gerlai, 2013b). Zebrafish were decapitated immediately following the acute ethanol exposure without prior anesthesia and their brains stored at –80 °C. All zebrafish handling and experimental procedures were in accordance with the guidelines of the Canadian Council on Animal Care (CCAC) and were approved by the Local Animal Care Committee (LACC).

2.3. Behavioral analysis

Video cameras were set up in front of the testing tanks and recordings were taken during both the novel tank exposure and acute ethanol exposure sessions. The video files were replayed and analyzed using EthoVision XT 8.5 (Noldus Info Tech, Wageningen, The Netherlands), an automated video tracking software. For the novel tank exposure, total distance traveled (cm, a measure of locomotor activity) was quantified for the entire 10 min duration. For the acute ethanol exposure, total distance traveled, distance to bottom (a measure of bottom dwelling), variance of distance to bottom (a measure of tank exploration) and absolute turn angle (a measure of erratic movement) were quantified in the last 10 min of exposure. We chose to analyze this specific period of time rather than the entire time-course of behavioral responses since the locomotor stimulant effects of ethanol are expected to have reached its peak after approximately 20 min (Tran and Gerlai, 2013b) and this would also allow zebrafish to acclimate to the environment. These behavioral measures were chosen because they are often utilized in the analysis of motor activity as well as anxiety and fear responses in the zebrafish (Bencan and Levin, 2008; Levin et al., 2007; Nowicki et al., 2014).

2.4. High precision liquid chromatography

The levels of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin, and 5-hydroxyindoleacetic acid (5-HIAA) from zebrafish brain tissue were quantified by high precision liquid chromatography (HPLC) using a previously established method (Chatterjee and Gerlai, 2009; Chatterjee et al., 2014; Gerlai et al., 2009a). The dopaminergic and serotonergic neurotransmitter systems are known to mediate locomotor activity as well as anxiety-like behaviors in zebrafish (Connors et al., 2014; Irons et al., 2013; Maximino et al., 2013a; Tran et al., 2014). Whole brains were dissected and sonicated in 10 µL of artificial cerebral spinal fluid containing 25 µM ascorbic acid. 1 µL of the homogenate was used to determine protein concentration using BioRad protein assay reagent (BioRad, Hercules, CA, United States).

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