Original Paper

Behavioral profile of adult zebrafish acutely exposed to a selective dopamine uptake inhibitor, GBR 12909

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Abstract

Background: The dopamine transporter (DAT) is the main regulator of dopamine concentration in the extrasynaptic space. The pharmacological inhibition of the DAT results in a wide spectrum of behavioral manifestations, which have been identified so far in a limited number of species, mostly in rodents.

Aim: Here, we used another well-recognized model organism, the zebrafish (*Danio rerio*), to explore the behavioral effects of GBR 12909, a highly-affine selective DAT blocker.

Methods: We evaluated zebrafish locomotion, novelty-related exploration, spatial cognition, and social phenotypes in the novel tank, habituation and shoaling tests, following acute 20-min water immersion in GBR 12909.

Results: Our findings show hypolocomotion, anxiety-like state, and impaired spatial cognition in fish acutely treated with GBR 12909. This behavioral profile generally parallels that of the DAT knockout rodents and zebrafish, and it overlaps with behavioral effects of other DAT-inhibiting drugs of abuse, such as cocaine and D-amphetamine.

Conclusion: Collectively, our data support the utility of zebrafish in translational studies on DAT targeting neuropharmacology and strongly implicate DAT aberration as an important mechanisms involved in neurological and psychiatric diseases.

Keywords

Zebrafish, dopamine transporter, hypolocomotion, spatial cognition, novel tank test, shoaling

Introduction

Dopamine, a major brain neurotransmitter, is crucial for controlling human and animal locomotion, cognition, motivation, and reward (Girault and Greengard, 2004; Klein et al., 2019). For example, aberrant dopaminergic control of reward circuits is a major cause of addiction (Volkow et al., 2011), which is commonly associated with altered extracellular dopamine levels (Salimpoor et al., 2011; Wise and Robble, 2020), especially in striatum and nucleus accumbens (NAcc) (Salimpoor et al., 2011). In contrast, reduced dopamine signaling plays a role in the pathogenesis of mood disorders, underlying motor retardation (Masato et al., 2019), anhedonia (Belujon and Grace, 2017), and dysthymia (Ishizaki and Mimura, 2011). Stress, in turn, can also modulate mesolimbic dopamine reward circuits, thereby pathogenetically bridging addiction (Sim et al., 2013) and affective disorders (Baik, 2020).

Drugs that affect central dopaminergic signaling include dopamine receptors agonists and antagonists, dopamine-releasing agents, dopamine precursors, related enzymatic cofactors and inhibitors, various toxins, as well as enhancers or inhibitors of its reuptake mediated by the dopamine- (DAT) and the vesicular monoamine transporters. Dopaminergic drugs are efficient in treating a wide range of brain disorders, including Parkinson's disease (dopamine agonists + L-DOPA) (Wachtel, 1991), schizophrenia and bipolar disorder (antipsychotics) (Serafini et al., 2022), or epilepsy (e.g., dopamine D1 and D2 receptor agonists) (Bozzi and Borrelli, 2013; Brodovskaya and Kapur, 2021). At the same time, commonly abused drugs cocaine, amphetamine, methamphetamine, adderall, ritalin, and wellbutrin all promote addiction by inhibiting DAT (Vaughan and Foster, 2013; Verma, 2015) and elevating striatal dopamine release (Oleson et al., 2009).

In the search of dopaminergic drugs that increase synaptic dopamine but not dopamine release, GBR 12909 (vanoxerine) was found to be more affine to DAT than cocaine (Izenwasser et al., 1990). Some evidence suggests GBR 12909 as a promising antidepressant devoid of adverse cocaine-like behavioral effects

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in clinical studies (Preti, 2000; Søgaard et al., 1990). However, acutely administered GBR 12909 induces hyperactivity and anxiety in mice (Bigot et al., 2022), similarly to conventional DAT blockers (Hirate and Kuribara, 1991), whereas other animal studies show that GBR 12909 attenuates cocaine self-administration (Rothman et al., 2008).

Given the potential therapeutic value of GBR 12909 and similar drugs, but their poorly understood central effects in both humans and mammals, other vertebrate model species may be beneficial to address this problem further. For example, a small freshwater teleost fish, the zebrafish (*Danio rerio*), is rapidly gaining utility in translational neuroscience research and preclinical central nervous system (CNS) drug screening, due to its sufficient genetic, physiological, neurochemical, neuroanatomical, and behavioral similarity to mammals (Howe et al., 2013; Kozol et al., 2016). Zebrafish also possess well-developed, evolutionarily conserved dopaminergic system (Stewart et al., 2015) and are highly sensitive to a wide range of conventional dopaminergic drugs, including antipsychotics (e.g., haloperidol, clozapine, olanzapine, risperidone, buspirone) (Bruni et al., 2016), cocaine (Darland and Dowling, 2001; López-Patiño et al., 2008), D1- and D2-like selective agonists (e.g., SKF-38393 and quinpirole) (Irons et al., 2013), and substances promoting the loss of dopaminergic neurons (e.g., 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 6-hydroxydopamine) (Wasel and Freeman, 2020). In contrast, GBR 12909 investigated here affects dopamine transmission via inhibiting DAT manifoldly in comparison with other DAT blockers (e.g., cocaine) (Izenwasser et al., 1990), along with dopamine release suppression (Singh, 2000). Here, we used this powerful model organism to characterize the acute effects of GBR 12909 on zebrafish behavioral phenotypes. Specifically, we evaluated the effects of GBR 12909 in adult zebrafish subjected to the novel tank test (NTT) (Haghani et al., 2019), the habituation-based spatial working memory test (Wong et al., 2010) and the shoaling test (ST) (Facciol and Gerlai, 2020), to assess their general locomotor, anxiety-like, and social behaviors.

Materials and methods

Animals and housing

A total of 120 adult heterogenous wild-type short-fin outbred zebrafish (4–6 months old, ~50:50 male:female ratio) were obtained from a commercial distributor (Axolotl, Ltd., St. Petersburg, Russia) and acclimated for 3 weeks prior to the experiments in two 80-L plastic tanks with filtered oxygenized water maintained at 25°C–27°C and pH = 7.2–7.4, treated with AquaSafe™ (Tetra, Ltd., Melle, Germany) and methylene blue (Medosa, Ltd., Moscow, Russia). Illumination (800–900 lx) was provided by ceiling-mounted fluorescent light tubes on a 12-h schedule (8 am—lights on, and 8 pm—lights off). Fish were fed twice a day with TetraMin commercial flake fish food (Tetra, Ltd., Melle, Germany).

All animals used in the present study were experimentally naïve, and none of them were excluded from the analyses. Husbandry adhered to the guidelines for zebrafish care (Westerfield, 2000) as well as the national and institutional guidelines and regulations. The study, experimental design, and its description here, as well as data analysis and presenting, adhered to the Animal Research: Reporting of In Vivo Experiments guidelines for reporting animal research, the 3Rs (Replacement-Reduction-Refinement) principles of humane animal experimentation, and the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence guidelines for planning animal research and testing. The outbred strain selection for the present study was based on population validity considerations and their relevance for the present study. Briefly, although genetically controlled models (e.g., inbred zebrafish strains) can be a better reproducible and more reliable system for neurogenetics research, modeling CNS disorders, such as in the present study, involves "real" human disorders affecting genetically heterogenous populations. Thus, using outbred populations of zebrafish (such as selected here) was deemed a more populationally valid and translationally relevant approach for the purpose of this study (De Abreu et al., 2021).

Pharmacological manipulations

All fish were exposed acutely to either drug-free water or to 0.25 (0.1 for ST), 0.5, and 1 mg/L GBR 12909 dihydrochloride (≥98% HPLC grade, Sigma-Aldrich, Merck, USA) for 20 min prior to behavioral testing. The drug was dissolved in fresh dechlorinated water, and its doses and the duration of treatment were chosen based on our own pilot study confirming the absence of non-specific toxic/sedative effects of this drug. Drug treatment was performed in a 750-mL beaker filled with 350 mL of drug solution at desired dose, which was refreshed after every three fish. In ST, each 5-fish shoal was exposed to the drug in 1.5-L tanks and video-recorded, as described below.

Behavioral assessment

Following acute GBR 12909 exposure, zebrafish general activity, novelty-evoked anxiety-like behavior, spatial cognition, and social behavior were assessed in the NTT, intrasession habituation, and ST, similar to previously published studies (Cachat et al., 2011b; Facciol and Gerlai, 2020; Wong et al., 2010). Each test was performed on a separate cohort of zebrafish, to avoid the test battery effect. Prior to experimental manipulations, all fish were acclimated for 1.5 h to the testing room. All behavioral paradigms used plastic rectangular 1.5-L tanks (17 height × 19.5 width × 4.5 length, cm) filled with water. Three sidewalls (back and lateral) of these tanks were covered with white adhesive paper to increase the contrast. Illumination in experimental area was similar to housing conditions (800–900 lx). Prior to experimental manipulations, all fish were acclimated for 1.5 h to the testing room. Behavioral procedures were conducted between 11:00 and 16:00 h, and videos were recorded using Logitech C270 HD 720p web-cameras (Logitech, Plc., Lausanne, Switzerland) at 30 frames/s.

The NTT apparatus was divided by dotted horizontal line into two equal top and bottom zones. Prior to testing, all fish were experimentally naïve and divided into four groups ($n = 15$ per group), including control and 0.25, 0.5, and 1 mg/L GBR 12909 groups. Each fish was recorded individually for 5 min, scoring general locomotor indices, such as total distance moved (m), mean velocity (m/s), maximum velocity (m/s), maximum acceleration (m/s^2), rotations, not-moving bouts (episodes without directed movements, ≤0.02 m/s) and duration (s), and high-velocity bouts (≥0.1 m/s) and duration (s). The analysis of zone-specific locomotion included distance moved in top (m), maximum velocity in top (m/s), distance moved in bottom (m), and maximum velocity in

bottom (m), similar to (Suryanto et al., 2022). Anxiety-related behavioral endpoints included meandering (deg/m), cumulative turn angle (deg), mean angular velocity (deg, s), top entries (top frequency), time spent in top (s), top latency (s), bottom latency (s), total activity, and zone (top/bottom)-related activity, as in shown by Cachat et al. (2011c). All endpoints were analyzed using automated video-tracking EthoVision XT17 software (Noldus IT, Wageningen, Gelderland, Netherlands).

Intrasession habituation was analyzed using the 5-min NTT data, assessing a single-minute habituation ratio (SHR) by comparing that at the first vs the last (5th) minute, and cumulative habituation ratio (CHR) by comparing first vs second halves of the test (Raymond et al., 2012) for total distance moved (m), top/bottom distance moved, high-velocity (not-moving) bouts and duration (s), top frequency, top duration (s), and top/bottom activity.

The ST involved a 3-min acclimation period followed a 10-min video-recording of fish group behavior in a separate cohort of experimentally naïve fish with three 5-fish shoal ($n = 15$ per group) with 33 screenshots per group (11 screenshots per shoal, 1 screenshot per min for minutes 0–10), scoring average inter-fish distance (cm), closest and farthest neighbor distances (cm) between the fish, shoal area (cm^2), and the number of top dwelling fish (expressed as % of total) (Pham et al., 2012), using the ImageJ software (NIH, Bethesda, MD, USA), similar to (Kim et al., 2017). Shoal area was calculated by this software as the square of the area formed by consecutive straight lines connecting each fish location on the screenshot, according to Rosa et al. (2020). In all the analyses used here, the individual fish location was determined computationally as the body center point.

Statistical analyses

Statistical analyses were performed by GraphPad Prism 8 (GraphPad, San Diego, CA, USA), assessing data normality by a combination of Anderson-Darling, D'Agostino-Pearson, Shapiro-Wilk, and Kolmogorov-Smirnov tests. Since most of the data were not distributed normally, our analyses utilized nonparametric Kruskal–Wallis (KW) test, followed by Dunn's post-hoc testing for significant KW, NTT and ST data. SHR and CHR habituation indices were analyzed using the Wilcoxon matched-pairs signed rank *U*-test. NTT and ST results are presented as median with interquartile range. Habituation results are presented as mean ± SEM. A sample size ($n = 15$) chosen for each behavioral test here was calculated by the G*Power software (Heinrich Heine University, Düsseldorf, Germany) by point-biserial correlation (effect size ρ 0.62, statistical power 0.8, and two-tailed $p = 0.05$), and was also based on the results from our own pilot studies with GBR 12909, as well as on our previous studies screening CNS drugs and novelty-evoked behavior in zebrafish (Bozhko et al., 2022; Cachat et al., 2010).

Results

The novel tank test

The NTT experiment revealed overt hypolocomotion in fish treated by 1 mg/L GBR 12909, with significant differences in several indices, such as total distance moved and mean velocity ($p < 0.0001$ vs control, $p < 0.001$ vs 0.25 mg/L, and $p < 0.01$ vs 0.5 mg/L), maximal velocity ($p < 0.01$ vs control, $p < 0.05$ vs 0.5 mg/L) and maximal acceleration ($p < 0.01$ vs control, $p < 0.05$ vs 0.25 mg/L, and $p < 0.01$ vs 0.5 mg/L, Figure 1(a)). Fish exposed to 1 mg/L GBR 12909 spent longer time without directed moving versus other groups ($p < 0.0001$ vs control, $p < 0.001$ vs 0.25 mg/L, and $p < 0.01$ vs 0.5 mg/L), while the frequency of "not moving" was higher only in 0.25 mg/L group ($p < 0.05$) vs controls. The 1 mg/L group had fewer high-velocity bouts and shorter high-velocity locomotion time than control ($p < 0.01$, $p < 0.001$) and 0.5 mg/L groups ($p < 0.05$ both). Fish exposed to 0.25 mg/L of the drug did not differ from any other group in high-velocity measures (Figure 1(a)). Total locomotor activity significantly differed at 1 mg/L from control and 0.25 mg/L groups ($p < 0.0001$ both), whereas 0.25 and 0.5 mg/L groups rotated more frequently than the 1 mg/L-treated fish ($p < 0.05$ and $p < 0.01$, respectively), who meandered more than all other groups ($p < 0.001$ vs control, $p < 0.01$ vs 0.25 and 0.5 mg/L, Figure 1(a)). Cumulative turn angle and mean angular velocity did not reveal significant differences.

The vertical activity analyses showed increased bottom dwelling in 1 mg/L-treated fish (less top frequency $p < 0.0001$ and duration $p < 0.0001$, longer top latency $p < 0.001$) compared to controls. While the 0.25 mg/L group entered the top more frequently ($p < 0.001$) and dwelled there longer ($p < 0.01$) than fish exposed to 1 mg/L (Figure 2), control also swam longer (Figure 1(b)) and spent more time in the top (Figure 2) than 0.5 mg/L group ($p < 0.05$, $p < 0.01$). Assessing maximal velocity in the top, controls and 0.25 mg/L fish swam faster than 1 mg/L -treated zebrafish ($p < 0.0001$, $p < 0.05$, Figure 1(b)). Overall, controls and 0.25 mg/L fish were more active in the top than the 1 mg/L group ($p < 0.0001$, $p < 0.001$), while controls were also more active in the top than 0.5 mg/L group ($p < 0.05$, Figure 2).

The per-zone locomotion analyses supported general hypolocomotion of 1 mg/L fish and lesser bottom dwelling in control fish (Figure 1(b)), while total bottom activity was higher in control ($p < 0.05$) and 0.25 mg/L ($p < 0.0001$) fish than in 1 mg/L group (Figure 2). Specifically, 0.25 mg/L ($p < 0.05$) and 0.5 mg/L ($p < 0.01$) fish covered longer distance in the bottom zone than control and 1 mg/L groups. Additionally, control and 0.5 mg/L fish showed higher maximal velocity than 1 mg/L-treated fish (both $p < 0.05$, Figure 1(b)).

Intrasession habituation

While GBR 12909 at 1 mg/L significantly reduced zebrafish habituation, its smaller doses (0.25 and 0.5 mg/L) unaltered top frequency and top total activity SHR and CHR indices (Supplemental Table S1), as well as distance moved in the top (SHR, CHR $p < 0.05$; SHR $p < 0.01$, CHR $p < 0.05$, respectively), whereas total bottom activity habituated only in control fish (SHR $p < 0.001$, CHR $p < 0.05$). Habituation test revealed significant effect of GBR 12909 on general locomotion, including poorer habituation in all drug-treated groups, but not in controls fish (SHR $p < 0.0001$, CHR $p < 0.001$). However, a time-dependent increase in locomotor activity was observed in 0.25 mg/L-treated fish by reduced not-moving bouts (SHR $p < 0.05$, CHR $p < 0.05$), 0.5 mg/L by total distance (SHR, CHR $p < 0.05$), high-velocity duration (SHR $p < 0.05$), "not-moving" duration (SHR, CHR $p < 0.05$) and frequency (SHR $p < 0.05$), and 1 mg/L by lower not-moving duration (SHR $p < 0.05$, CHR $p < 0.05$, Supplemental Table S1).

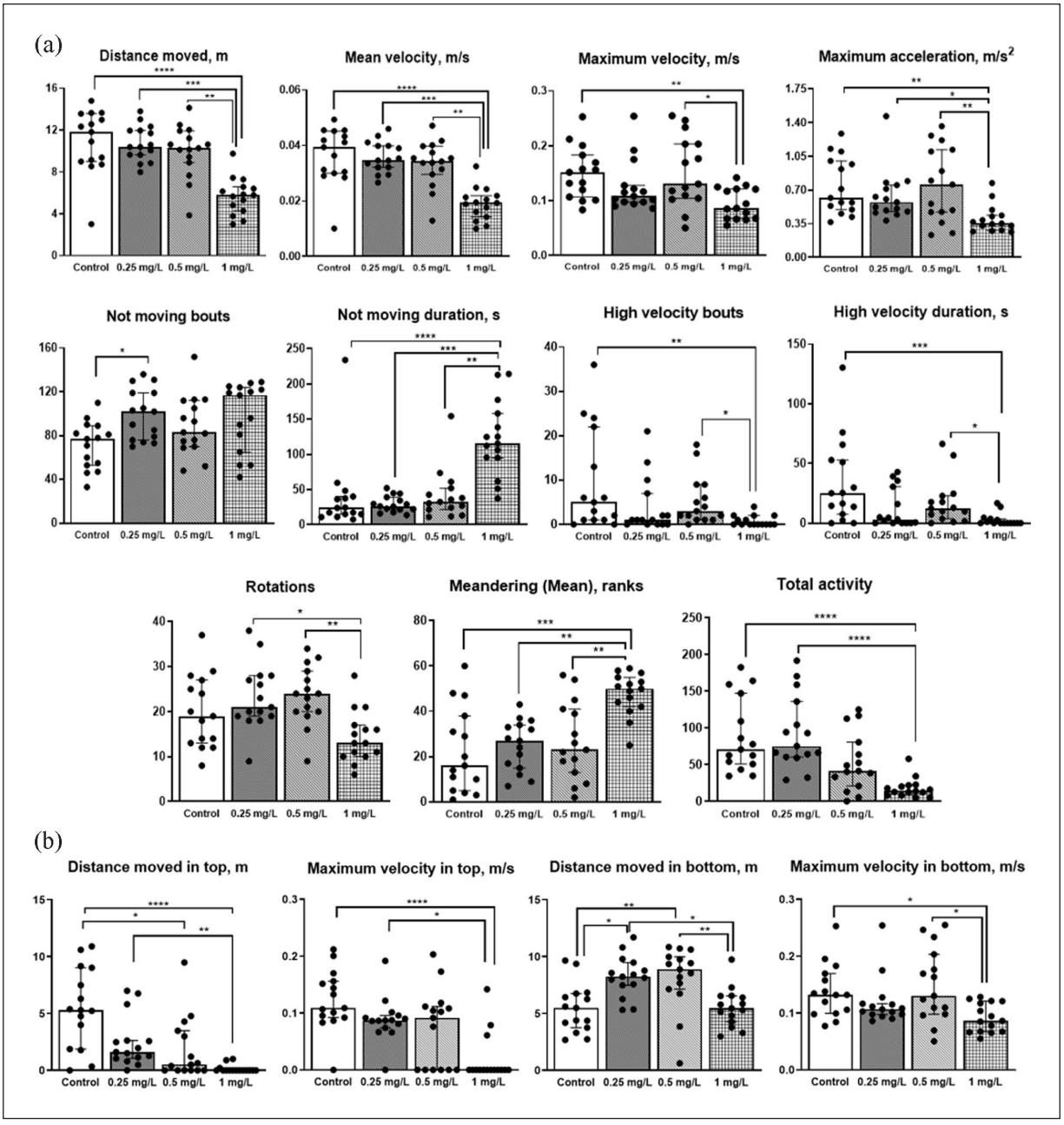

Figure 1. Zebrafish locomotion (a) and per-zone activity (b) endpoints assessed following an acute 20-min GBR 12909 treatment in the novel tank test; data are presented as median with interquartile range (n = 15 per group); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, Kruskal–Wallis (KW) test with post-hoc Dunn's correction.

Shoaling behavior

ST detected tighter shoals at 1 mg/L vs control and 0.1 mg/L groups (Figure 3), with shorter inter-fish distance ($p < 0.01$ vs control, $p < 0.0001$ vs 0.1 mg/L), nearest neighbor distance ($p < 0.01$ vs control, $p < 0.001$ vs 0.1 mg/L), and smaller shoal area ($p < 0.0001$ vs control, $p < 0.0001$ vs 0.1 mg/L). Fish treated with 0.1 mg/L also had longer average inter-fish distance, larger shoals than 0.5 mg/L fish (both $p < 0.001$), and longer farthest neighbor distance than 0.5 and 1 mg/L (both $p < 0.0001$) groups. The shoal area was also smaller in 1 vs 0.5 mg/L groups ($p < 0.01$). Finally, the 1 mg/L-exposed fish did not dwell in the top, whereas the 0.5 mg/L-treated fish showed fewer top-dwelling fish compared to control and 0.1 mg/L groups (both $p < 0.0001$, Figure 4).

Discussion

Dopamine extracellular levels and signaling are strongly controlled by DAT (Gregory and Bertha, 2005), whose dysregulation leads to various brain disorders, including addiction, attention deficit/hyperactivity disorder, bipolar disorder, and Parkinson's disease (Gregory and Bertha, 2005; Vaughan and Foster, 2013). Well-established animal genetic models often involve DAT

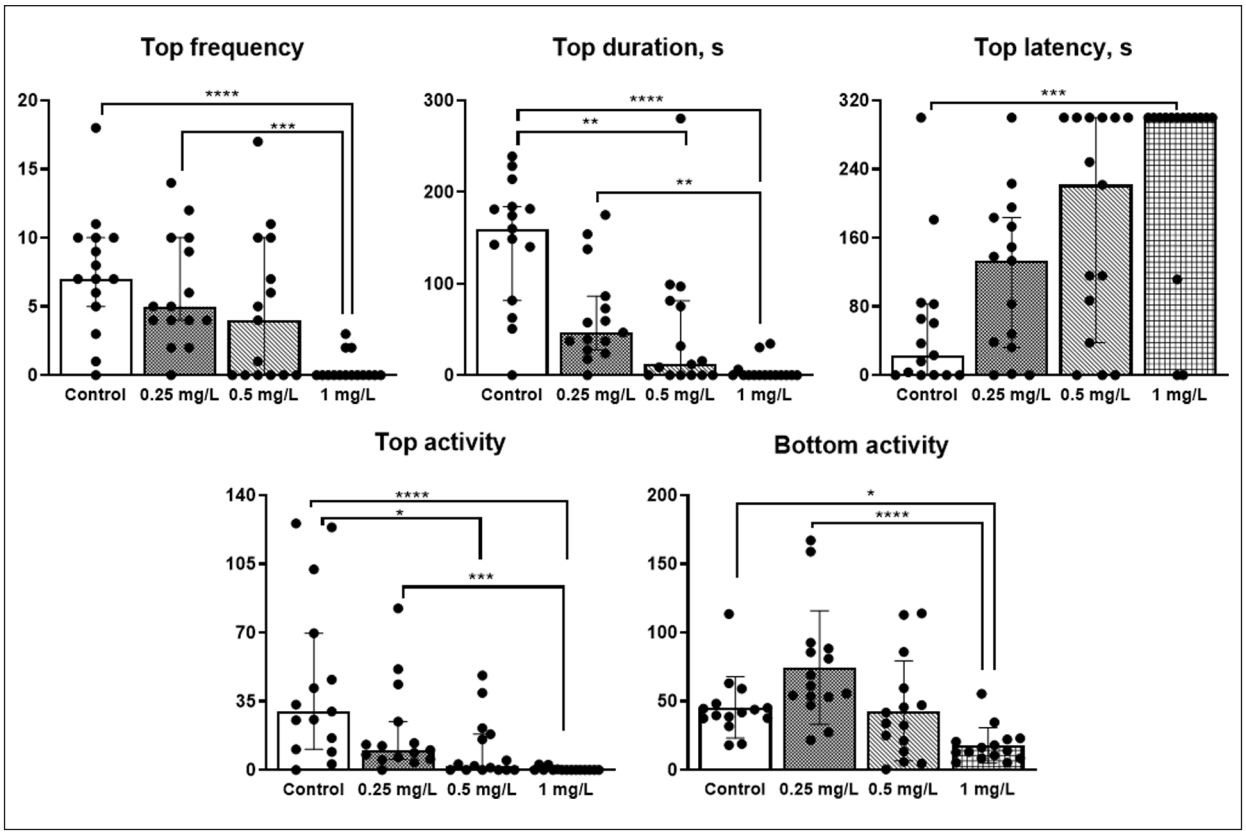

Figure 2. Zebrafish vertical activity endpoints assessed following an acute 20-min GBR 12909 treatment in the novel tank test; data are presented as median with interquartile range (n = 15 per group); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, Kruskal–Wallis (KW) test with post-hoc Dunn's correction.

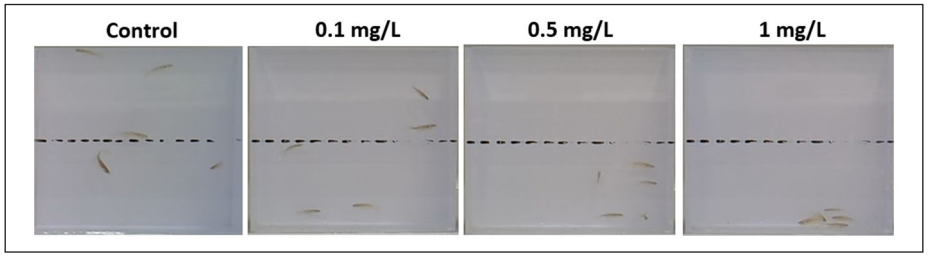

Figure 3. Representative images showing zebrafish shoals of zebrafish treated acutely for 20 min with GBR 12909, compared to water-treated control fish.

knockout rodents (Cinque et al., 2018), the phenotypes of which recapitulate dopaminergic neurodegenerative deficits and include impulsivity and perceptual and memory deficits (Efimova et al., 2016; Leo et al., 2018). Furthermore, pharmacological inhibition of DAT by amphetamine, cocaine, or GBR 12909 induces similar effects in mice with normal DAT function (Salahpour et al., 2008; Spielewoy et al., 2001), recapitulating some mania-related features (hyperlocomotion and elevated exploratory behavior) in bipolar depression patients (Young et al., 2010). The present study is the first attempt to explore the role of pharmacological inhibition of DAT in adult zebrafish.

Overall, zebrafish displayed overt hypolocomotion at the highest GBR 12909 dose tested here (1 mg/L), with a lesser trend for high-speed locomotion at the 0.25 mg/L dose vs control and the 0.5-mg/L fish groups. Interestingly, similar hypolocomotion is also demonstrated in zebrafish Y-maze for chronic treatment with another DAT inhibitor, D-amphetamine (Cleal et al., 2021b), likely representing an early-onset Parkinson's phenotype associated with DAT deficiency (Wang et al., 2019). Additionally, many Parkinsonic patients experience motor score decrease paralleling exponential or linear decline of DAT activity (Ikeda et al., 2019). Furthermore, clinical observations of PD-patients also revealed reduced DAT availability in striatum (right putamen) in the subgroup of Parkinsonic patients with elevated anxiety (Erro et al., 2012).

Lower vertical exploration indicates anxiogenic-like effect of 0.5 and 1 mg/L GBR 12909, since bottom dwelling is often associated with increased anxiety in zebrafish (Cachat et al., 2011b). Alternatively, lower locomotor and vertical activity at 1 mg/L may also reflect sedation in these fish (e.g., as observed with acute ethanol (Vossen et al., 2022)). However, fish treated with this GBR 12909 dose performed significantly more meandering

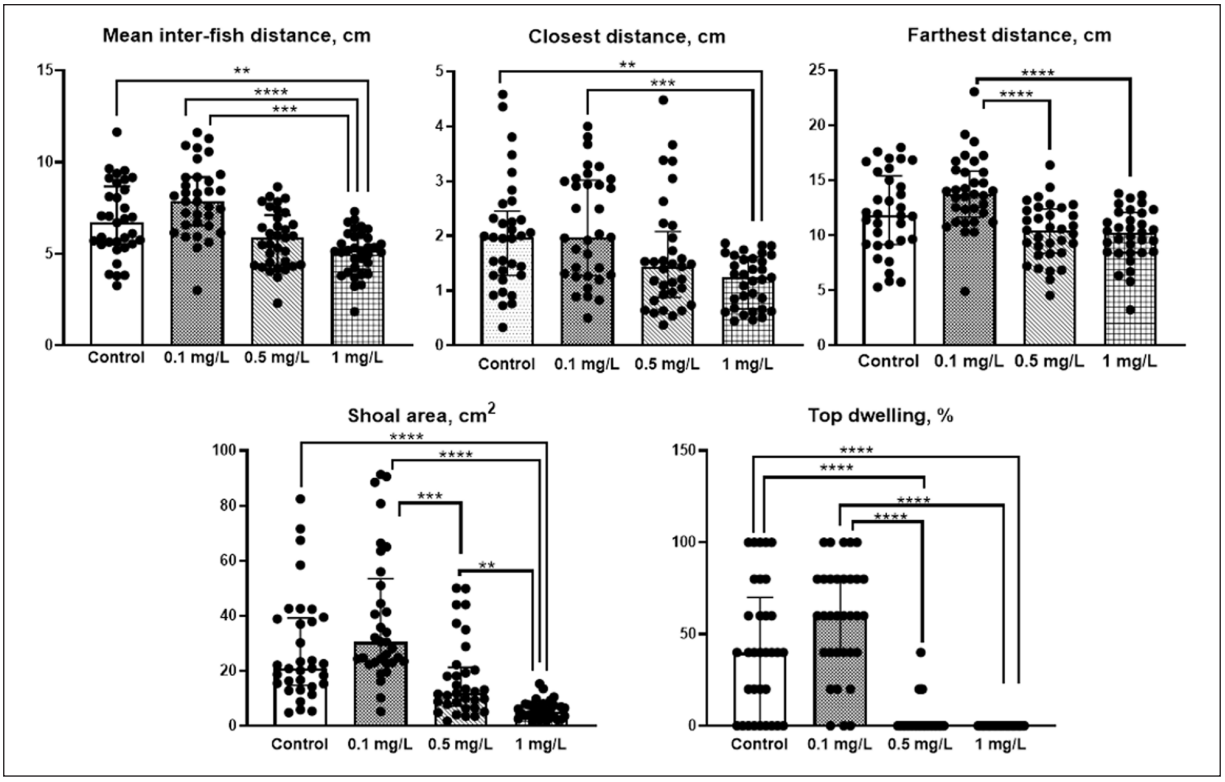

Figure 4. Behavioral effects of acute 20-min GBR 12909 treatment in adult zebrafish shoaling test; data are presented as median with interquartile range (n = 15 per group); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, Kruskal–Wallis (KW) test with post-hoc Dunn's correction.

than other groups (hence likely reflecting fish anxiety (Cachat et al., 2011a; Johnson and Hamilton, 2017), rather than sedation). Furthermore, 0.5 mg/L also evoked lower vertical activity without hypolocomotion (Figures 1 and 2), further supporting anxiety-like profile of the drug. Interestingly, the zebrafish DAT knockout also evokes elevated anxiety with bottom preference and thigmotaxis (Kacprzak et al., 2017), further consistent with an anxiogenic profile of GBR 12909 in the NTT observed here (Figure 1).

In neurobehavioral screening, intrasession habituation to novel environments represents an evolutionarily conserved cognitive phenotype (Wong et al., 2010) related to spatial working memory (Galani et al., 1998) and basic form of learning (Schmid et al., 2015), sometimes mediated by the mesolimbic and mesocortical dopaminergic neurotransmission (Lloyd et al., 2014). Assessing habituation is well-established and common in zebrafish models and tests, including the NTT-based CNS drug screens (Stewart et al., 2013). Poorer habituation in zebrafish treated by 1 mg/L GBR 12909, seen in the present study, strikingly recapitulates reduced spatial working memory reported in the DAT knockout mice (Li et al., 2010). Somewhat similar working memory decline is also observed in adult zebrafish with reduced DAT expression following a dopamine D1/D5 receptor agonist SKF-38393 treatment at young ages (Cleal et al., 2021a). Natural rise of zebrafish locomotion in the habituation test was reduced by GBR 12909 at all doses used here (Supplemental Table S1), paralleling similar effects of a relatively novel DAT inhibitor, CE-123, thus attenuating an alcohol-induced hyperactivity in rats (Gibula-Tarlowska et al., 2021). Furthermore, so-called locomotor habituation analyses are often included in the neurobehavioral studies (Brenes et al., 2009; Spielewoy et al., 2000), especially related to the open field tasks, highly relevant to short-term spatial cognition (Typlt et al., 2013; Willi et al., 2012).

Although GBR 12909 did not disrupt ST social behavior, the drug made zebrafish shoals tighter and increased their bottom preference (Figures 3 and 4). Since tighter shoals are common for anxiety in zebrafish (Golla et al., 2020), the effects of 1 mg/L GBR 12909 in ST seem to corroborate anxiogenic profile of this drug observed in the NTT. Likewise, reduced top dwelling (another sign of anxiety (Pham et al., 2012)) was also seen in both 0.5 mg/L and especially 1 mg/L GBR 12909 groups.

Considering potential toxic properties, GBR 12909 induced arrhythmogenic effects in halothane-anesthetized dogs via the inhibition of rapid potassium channels (I_{Kr}) with resulting intracellular Ca^{2+} overbalance (Hagiwara-Nagasawa et al., 2021). Although GBR 12909 also showed proarrhythmic potential in humans, indicating prolonged QTc interval on electrocardiogram (Rothman et al., 2008), clinical GBR 12909 safety studies did not reveal behavioral alterations in humans, unlike other DAT blockers (e.g., cocaine) (Preti, 2000; Tella et al., 1996). However, acute GBR 12909 induced overt hypolocomotion in adult zebrafish, a profile in line with some genetic (e.g., rodent DAT knockout (Cinque et al., 2018; Spielewoy et al., 2001)) or pharmacological (e.g., amphetamine-treated rodents (Cinque et al., 2018; Sukhanov et al., 2019) and zebrafish (Cleal et al., 2021b)) models of DAT deficits. Furthermore, GBR 12909-induced hypolocomotion in zebrafish is unlikely due to putative cardiotoxicity, as no

data from studying other cardiotoxic agents link hypolocomotion and cardiotoxicity (Heideman et al., 2005; Wiprich et al., 2020; Yang et al., 2022). Moreover, no visible signs of ataxia have been observed in the 1 mg/L group in high-resolution (hd – 1280 × 720) videos in the present study.

Unlike D-amphetamine improving spatial cognition in the zebrafish Y-maze (Cleal et al., 2021b), GBR 12909 impaired their habituation in the present study (Supplemental Table S1), similar to the DAT knockout mice (Efimova et al., 2016). Furthermore, GBR 12909 treatment evokes anxiety-like phenotype in zebrafish NTT and ST, again being consistent with data on increased anxiety in rodents treated with GBR 12909 (Bigot et al., 2022; Young et al., 2010). However, the fact that acute GBR 12909 evokes hypolocomotion, anxiety, and cognitive deficit in zebrafish here (Figures 1–3), but not clinically (Preti, 2000; Rothman et al., 2008; Tella et al., 1996), calls for further clinical and preclinical studies of this drug, including both chronic treatment and withdrawal paradigms.

Conclusion

Overall, GBR 12909 evoked unique behavioral phenotype in zebrafish previously not observed in rodent models. GBR 12909 at a high dose induces hypolocomotion unlikely related to general and cardiological toxicity. Although DAT knockout rodents display hyperactivity, GBR 12909-induced locomotor decline in zebrafish resembles clinical data on reduced locomotion accompanied by DAT decrease in Parkinsonism (Ikeda et al., 2019). Moreover, generally anxiogenic traits observed in NTT and ST in zebrafish groups treated with GBR 12909 at 1- and 0.5 mg/L doses parallel clinical data on comorbidity of anxiety and Parkinson's disease (Erro et al., 2012). Finally, intrasession habituation analysis revealed likely impaired spatial cognition and locomotor habituation, evoked by a high dose of GBR 12909.

In general, our findings support the utility of zebrafish in preclinical studies on DAT neuropharmacology and DAT-related neuropsychiatric disorders. However, several limitations of this study include its use of only behavioral methods, albeit empowered by sophisticated computational analyses. Likewise, it yet remains to be clarified whether the highest GBR 12909 dose used here may induce some CNS or general toxicity, especially given clinically observed proarrhythmic properties of GBR 12909 (Rothman et al., 2008). The temporal dynamics of GBR 12909 action, its chronic effects, putative drug withdrawal responses, addictive potential, as well as the impact on dopamine metabolism, monoamine receptors, other neurotransmitter systems, and the expression of key brain genes, warrant further studies in both zebrafish and rodent preclinical models.

Declaration of conflicting interests

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Supplemental material

Supplemental material for this article is available online.

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