Progress in Neuropsychopharmacology & Biological Psychiatry 108 (2021) 110179

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Feeding status alters exploratory and anxiety-like behaviors in zebrafish larvae exposed to quinpirole

Débora Dreher Nabinger [a], Stefani Altenhofen [a,b], Júlia Vasconcellos Peixoto [a], Julia Maria Kuhl da Silva [a], Robert Gerlai [c], Carla Denise Bonan [a,b,d,*]

[a] Laboratório de Neuroquímica e Psicofarmacologia, Programa de Pós-Graduação em Biologia Celular e Molecular, Escola de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] Programa de Pós-Graduação em Medicina e Ciências da Saúde, Escola de Medicina, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Department of Psychology, University of Toronto Mississauga, ON, Canada
[d] Instituto Nacional de Ciência e Tecnologia em Doenças Cerebrais, Excitotoxicidade e Neuroproteção, Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:
Behavior
Nutritional state
Quinpirole
Zebrafish larvae

ABSTRACT

The dysfunction of dopaminergic signaling is associated with several neurological disorders. The use of pharmacological agents that interact with this signaling system may be employed to understand mechanisms underlying such disorders. Nutritional status can impact dopamine reuptake, receptor affinity, transporter activity, and the effects of drugs that bind to dopamine receptors or interact with dopaminergic system. Here we evaluated the effects of quinpirole (a dopamine D2/D3 receptor agonist) exposure on fed and non-fed zebrafish larvae. Zebrafish larvae (6 days post-fertilization, dpf) were exposed to quinpirole (5.5, 16.7, and 50.0 μM) or water (control group) for one hour. To evaluate the effect of feeding status on quinpirole exposure, the experiments were performed on fed and non-fed animals, a between subject experimental design. Both fed and non-fed quinpirole treated larvae exhibited increased erratic movements compared to controls in an open tank exploration task. No alterations were observed on the main parameters of exploratory behavior and swim activity for non-fed larvae treated with quinpirole compared to controls. However, fed animals exposed to quinpirole exhibited increased locomotor activity, anxiety-like behavior, and repetitive circular movements when compared to controls and non-fed exposed animals. In addition, we observed quinpirole exposure to have no effects on morphological parameters and heartbeat, but to impair optomotor responses in both fed and non-fed larvae compared to control. We also found quinpirole effects to interact with feeding status, as quinpirole-treated fed larvae improved while quinpirole treated non-fed larvae impaired their avoidance reaction towards an aversive stimulus. These results indicate that the behavioral effects of quinpirole exposure depended upon feeding status. They showed that consumption of food, a naturally rewarding stimulus known to engage the dopaminergic system, made this neurotransmitter system more susceptible to quinpirole's effects.

1. Introduction

Dopaminergic signaling is known to regulate a wide array of cerebral functions related to motor activity, fear, mood, attention, cognition, learning, and memory (Goldman-Rakic, 1998; Bjorklund and Dunnett, 2007; Jones and Miller, 2008; Beaulieu and Gainetdinov, 2011). Dysfunction in dopaminergic signaling is associated with several psychiatric, neurodegenerative and neurobehavioral disorders, including Alzheimer's disease, Parkinson's disease, schizophrenia, Tourette syndrome, and attention deficit hyperactivity disorder, as well as drug and alcohol abuse related disorders (Howes et al., 2015; D'Amelio et al., 2018; Burns et al., 2019; Chadehumbe and Brown, 2019; Klein et al., 2019; Armstrong and Okun, 2020). Dopamine effects are mediated by two families of G protein-coupled receptors, D1-like receptor family (D1 and D5) and D2-like receptor family (D2, D3, and D4). Through signaling events mediated by these receptors, dopamine can govern the initiation and execution of movement (Beaulieu and Gainetdinov, 2011) as well as numerous other behavioral functions.

* Corresponding author at: Laboratório de Neuroquímica e Psicofarmacologia, Escola de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil.
E-mail address: cbonan@pucrs.br (C.D. Bonan).

https://doi.org/10.1016/j.pnpbp.2020.110179
Received 3 July 2020; Received in revised form 11 November 2020; Accepted 12 November 2020
Available online 16 November 2020

To better understand the functioning of this neurotransmitter system and the disorders involved, pharmacological agents that interact with dopaminergic signaling have been successfully utilized. Quinpirole is a psychoactive drug that acts as a selective agonist of dopamine D2 and D3 receptors. As an agonist of dopamine receptors, quinpirole modulates bioavailability of dopamine. Quinpirole administration in animal models induces several behavioral alterations. The most common include changes in locomotor activity, induction of stereotypical responses, elevated erratic and repetitive movements and changes in learning and memory processes (Irons et al., 2013; D'Angelo et al., 2014; Naderi et al., 2016a, 2016b; Bortolato and Pittenger, 2017; Abounoori et al., 2020). This agonist is often employed in animal models to study mechanisms underlying neurological disorders, including anxiety, schizophrenia and obsessive-compulsive disorder (Brown et al., 2012; D'Angelo et al., 2014; Archer and Kostrzewa, 2016; Stuchlik et al., 2016; Bortolato and Pittenger, 2017; Szechtman et al., 2017).

The functioning of the dopaminergic neurotransmitter system can also be affected by nutritional status (Briguglio et al., 2018). For example, feed status, amount of food intake, and proportion of nutrients are thought to affect several processes, including dopamine reuptake, receptor affinity, and the activity of dopamine transporters. The effects of pharmacological agents that bind to dopamine receptors or interact with biochemical mechanisms associated with the dopaminergic system are also affected by nutritional status (Patterson et al., 1998; Carr, 2002; Huang et al., 2006; Sevak et al., 2008; South and Huang, 2008; Thanos et al., 2008; Baladi and France, 2009).

Zebrafish has become a favored animal model for biomedical studies. This vertebrate offers unique advantages for the study of several biological processes. These include its external and rapid development, the high degree of nucleotide sequence homology between zebrafish and human genes, biochemical similarities in zebrafish and human signaling pathways, and its complex behavioral repertoire (Lele and Krone, 1996; Howe et al., 2013; Kalueff et al., 2014; Stewart et al., 2014). The dopaminergic system is well characterized in the zebrafish. This neurotransmitter system begins to develop at 15–18 h post-fertilization (hpf), and by 4 days post-fertilization (dpf), all neuronal cells, and their projections are present (Rink and Wullimann, 2001, 2002; Boehmler et al., 2004, 2007; Li et al., 2007; Tay et al., 2011). Zebrafish dopamine receptors homologous for all mammalian subtypes have been identified (except for D5). Expression of genes encoding these receptors is detected by 30 hpf, and the receptors themselves are functional by 4 dpf (Boehmler et al., 2004, 2007; Li et al., 2007).

Since food is a positive reinforcer which engages reward mechanisms including the dopaminergic neurotransmitter system, and nutritional state may alter binding interactions between dopamine and its receptors, we investigated how feeding may interact with the effect of a drug known to bind to and affect dopamine receptors, quinpirole, a D2/D3 receptor agonist. We chose the zebrafish as subject of our analysis, because this species is particularly amenable to, and has been successfully utilized in, psychopharmacological analyses. Given the possible complexity of interactions between feeding and dopaminergic drug effects, we hypothesized that quinpirole exposure may lead to increased swimming activity and anxiety-like behavior, depending upon the feeding state of the zebrafish larvae. Thus, in this proof or principle study, we investigate effects of quinpirole on the behavior of zebrafish and attempt to demonstrate interaction of quinpirole effects with feeding by employing the drug for fed and non-fed zebrafish larvae.

2. Methods

2.1. Animals

Six-day post-fertilization (dpf) zebrafish larvae (*Danio rerio*) of the wild-type AB strain were used in the experiments. For breeding, female and males (1:2) were placed in breeding tanks (beach style design - Tecniplast, Italy) overnight and separated by a transparent barrier. After the adults spawned, viable embryos were collected and transferred to sterile Petri dishes, which were kept in an incubator at 28 °C on a 14/10 h light/dark cycle. Larvae were kept in maintenance water, reverse osmosis-filtered water whose salinity was reconstituted to reach 400–600 μS and pH 6.5–7.5 (ammonia <0.004 ppm, nitrite <1 mg/L, nitrate <50 mg/L, hardness 80–300 mg/L and chloride 0 mg/L), ideal levels for the species. The mortality, hatching rates, and general morphology of the embryos and larvae were monitored daily. Only larvae without morphological changes were used in behavioral evaluations to ensure that the behavioral effects were not confounded. All protocols were approved by the Institutional Animal Care Committee from Pontifícia Universidade Católica do Rio Grande do Sul (CEUA-PUCRS, permit number 8854) and comply with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA). This study was registered in the Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado - SISGEN (Protocol No. A3B073D).

2.2. Treatment

Quinpirole hydrochloride (Sigma-Aldrich, St. Louis, MO, USA - Q102) was dissolved in water to prepare a stock solution of 3.9 mM. The test solutions were prepared directly from this stock solution before use. At 6 dpf, zebrafish larvae were exposed to three different nominal concentrations of quinpirole (5.5, 16.7, and 50.0 μM) or water (0 control group) for one hour by immersion (Irons et al., 2013). During the treatment period, larvae were evaluated for exploratory behavior, and, at the end of the 1 h exposure, morphology, heartbeat, and optomotor behavior were also assessed (Fig. 1).

2.2.1. Feed condition

To evaluate the potential effect of feeding state on quinpirole exposure induced behavioral changes, the experiments were performed on fed and non-fed animals. In the fed group, from 4 dpf onward, larvae received commercial flakes (TetraMin Tropical Flake Fish®) three times a day supplemented with paramecium. In the non-fed group, larvae did not receive food at any time before quinpirole treatment and phenotypical quantification (Fig. 1). In other words, we employed a 2 × 4 between subject design, with feeding status having 2 levels and quinpirole concentration having 4 levels.

2.3. Morphological evaluation

For morphological evaluation, at the end of the 1 h quinpirole exposure, larvae ($n = 30$) were placed under a stereomicroscope (3×) and photomicrographs were taken. Body length, ocular distance, and surface area of the eyes were measured (software NIS-Elements D 3.2 for Windows, supplied by Nikon Instruments Inc.). Body length is defined as the distance from the center of the eyes to the tip of the tail bud. Ocular distance is defined as the distance between the inner edges of the two eyes, and the size of the eyes was measured as the total surface area of the eyes (Lutte et al., 2015; Altenhofen et al., 2017).

2.4. Heartbeat rate

Larvae had their heart rates monitored at the end of the quinpirole treatment period under the stereomicroscope by a blind observer. Briefly, treated larvae and controls were placed in Petri dishes, and their heart rates were monitored for 60 s ($n = 30$). This measurement was conducted three times for each animal. The mean of the three measurements were analyzed and considered for statistical analysis. For all procedures, the water temperature was kept stable at 28 °C by a thermoplate coupled to the stereomicroscope (Nabinger et al., 2018).

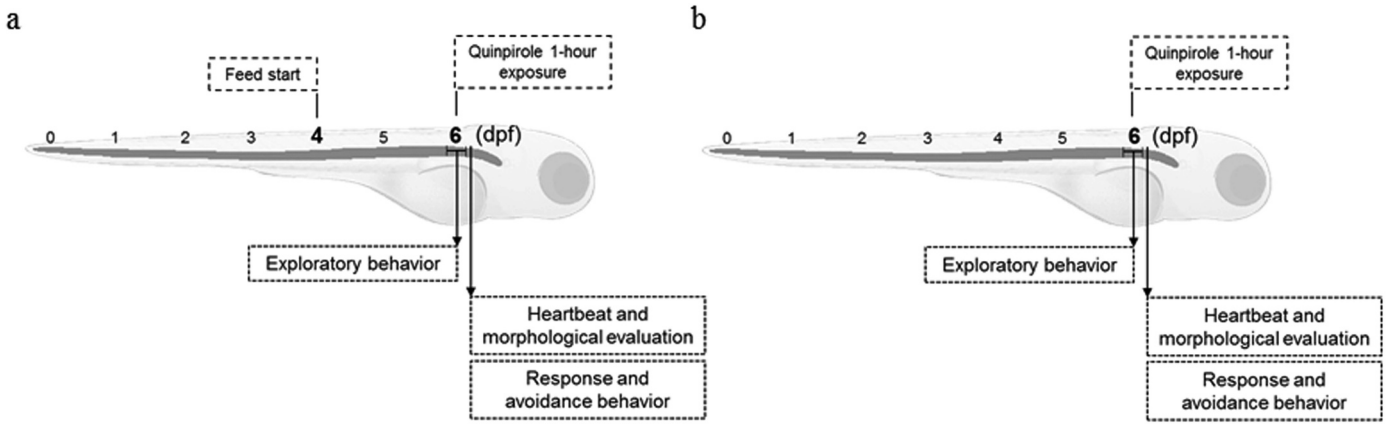

Fig. 1. Experiment flowchart. For fed group, larvae started receiving food from 4 dpf (a). Non-fed larvae did not receive food at any time (b). At 6 dpf, all larvae (fed and non-fed groups) were evaluated for heartbeat, morphology, and behavioral tasks.

2.5. Exploratory behavior

Larvae were individually placed in a 24-well plate, with each well as filled with appropriate quinpirole concentration or water, for a 1 h session of exploratory behavior analysis ($n = 24$) (Colwill and Creton, 2011; Irons et al., 2013). The performance was video recorded for automated analysis by Ethovision XT 10.0 software (30 frames per second video-sampling rate). Velocity (ratio between distance traveled and time mobile), time mobile, and acceleration were quantified and considered as measures of exploration of a new environment and swim activity. We also quantified absolute turn angle, which represents the change in direction of the center point of the animal between two consecutive samples irrespective of direction of turn and evaluates erratic movements. Last, we also quantified the time spent at the outer ring area of the well, thigmotaxis, as an indicator of anxiety-like behavior.

2.6. Optomotor behavior

2.6.1. Response behavior

To evaluate optomotor response behavior, larvae were placed in Petri dishes (15 larvae per dish, $n = 30$) over an LCD monitor and were exposed to a moving striped pattern, consisting of alternating black and white stripes (24.5 cm long and 1.5 cm wide) (a protocol adapted from Creton, 2009; Nery et al., 2017). The moving direction of stripes alternated every 1 min, with 5 s interval in which they faded before reappearing. The animated stripe presentation looped for a period of 12 min. For analysis, the dish area was virtually divided into two zones (up and down) and, at the end of each 1 min, the number of animals in each area was counted. This was considered indicative of their ability to respond (follow) the visual stimulus.

2.6.2. Avoidance behavior

For assessment of avoidance-escape from a visual stimulus (a 1.35 cm diameter red bouncing ball), larvae were placed in a 6-well plate (5 larvae per well, n = 30) over a LCD screen for a 5 min session following 2 min of habituation (Pelkowski et al., 2011). During the sessions, a red "bouncing ball" traveled from left to right over a straight 2 cm trajectory on the top half of the well area (stimulus area), which larvae could avoid by swimming to the lower half of the well. The number of larvae in the stimulus area during the 5 min session was considered indicative of deficit in the avoidance response.

2.7. Statistical analysis

Data from heart rate, morphological evaluation, optomotor and exploratory behaviors were analyzed by two-way ANOVA (with feed state (2 levels) and quinpirole concentration (4 levels) as between subject factors) followed by a post hoc Tukey's Honestly Significant Difference (HSD) test. For the analysis of exploratory behavior after fluoxetine exposure, two-way repeated measure ANOVA was used. Results are expressed as mean ± standard error of the mean (S.E.M). For all comparisons, the null hypothesis was rejected when its probability (p) was not more than 5% ($p \leq 0.05$).

3. Results

3.1. Morphological evaluation and heartbeat rate

In attempt to evaluate potential toxicological effects of quinpirole, analyses of gross morphology and heartbeat rate were performed at the end of the 1 h long exposure. There were no observable morphological alterations in treated larvae compared to control. No significant differences in body length, surface area of the eyes and ocular distance were found between controls and treated larvae or between the fed and non-fed groups (Fig. 2 a, b, c). Similarly, no significant changes in heart rate were observed (Fig. 2 d).

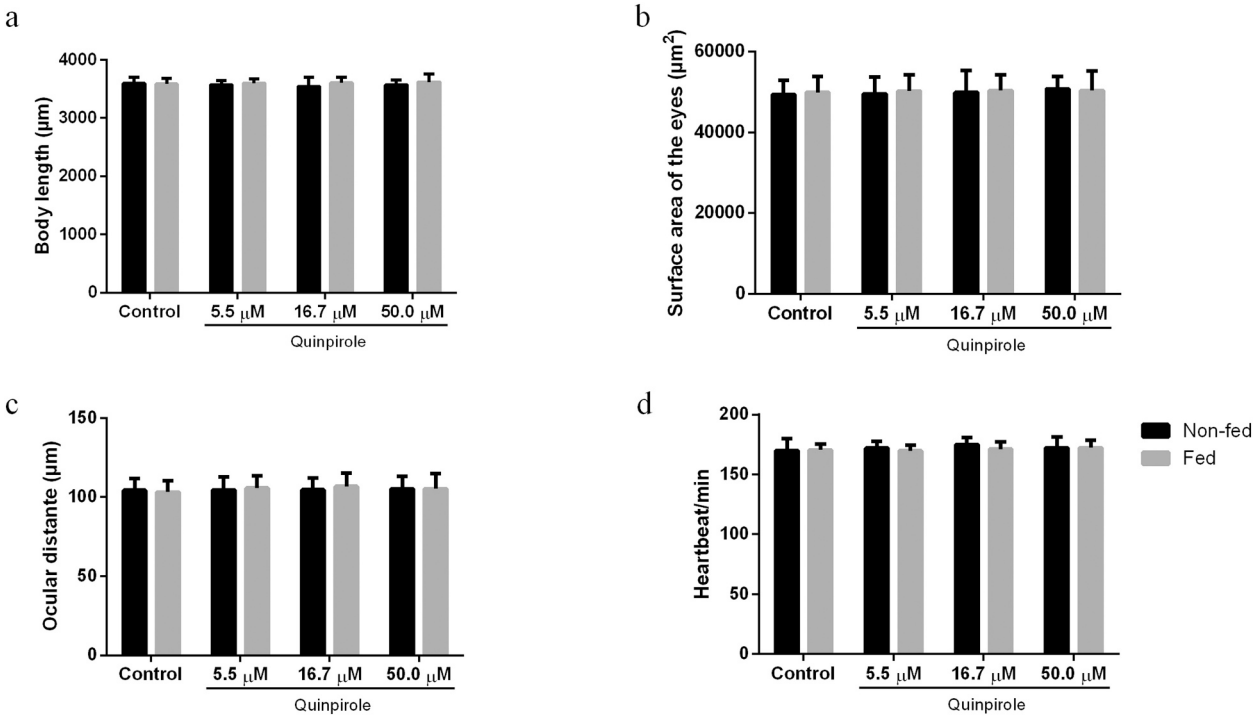

Fig. 2. Effects of quinpirole exposure on morphological parameters and heartbeat rate of 6 dpf zebrafish larvae. Body length (a), surface area of the eyes (b), ocular distance (c) and heartbeat rate (d) were evaluated after quinpirole exposure. Mean ± S.D. are shown. Sample sizes are $n = 30$ for each group. Black bars indicate non-fed group and grey bars indicate fed group. Note the lack of significant quinpirole exposure and feed status effects confirmed by Two-way ANOVA, followed by post-hoc Tukey's HSD test. For details of results of statistical analysis, see Results.

3.2. Exploratory behavior

The behavior of the larvae was evaluated during exposure to quinpirole in an open tank test environment (the well) without any stimulation. We quantified velocity, time mobile, acceleration, and parameter related to anxiety-like behavior.

Quinpirole treatment appeared to interact with feeding effects on activity parameters. While no alterations were observed on these parameters for non-fed larvae, fed larvae exposed to quinpirole increased their activity compared to fed control and non-fed quinpirole treated larvae (ANOVA, Quinpirole concentration: $F_{(3, 184)} = 4.507$; $p = 0.0045$. Feed status: $F_{(1, 184)} = 84.07$; $p < 0.0001$. Interaction: $F_{(3, 184)} = 5.181$; $p = 0.0018$). Increased velocity was observed in fed larvae exposed to 5.5 ($p < 0.0001$), 16.7 ($p = 0.0323$) and 50.0 μM ($p = 0.0008$) quinpirole compared to fed control. Furthermore, fed larvae exposed to 5.5 ($p < 0.0001$), 16.7 ($p = 0.0002$) and 50.0 μM ($p < 0.0001$) quinpirole exhibited elevated swimming speed compared to non-fed larvae exposed to corresponding quinpirole concentrations (Fig. 3 a).

The same pattern of results was observed for acceleration (ANOVA, Quinpirole concentration: $F_{(3, 184)} = 6.479$; $p = 0.0003$. Feed status: $F_{(1, 184)} = 41.57$; $p < 0.0001$. Interaction: $F_{(3, 184)} = 1.505$; $p = 0.2147$). Fed larvae exposed to 5.5 ($p = 0.0006$) and 50.0 μM ($p = 0.0138$) quinpirole increased their acceleration compared to fed control. Fed larvae exposed to 5.5 ($p = 0.0006$), 16.7 ($p = 0.0343$) and 50.0 μM ($p = 0.0042$) quinpirole also increased acceleration compared to non-fed larvae exposed to corresponding quinpirole concentrations (Fig. 3 b).

In the analysis of time mobile, quinpirole treatment was found non-significant, so was the interaction between quinpirole and feed status. However, feed status was found significant (ANOVA, Quinpirole concentration: $F_{(3, 184)} = 0.1949$; $p = 0.8998$. Feed status: $F_{(1, 184)} = 43.14$; $p < 0.0001$. Interaction: $F_{(3, 184)} = 1.094$; $p = 0.3531$). An increase of mobility in non-fed larvae exposed to 5.5 ($p = 0.0049$), 16.7 ($p = 0.0137$) and 50.0 μM ($p = 0.0016$) quinpirole compared to fed larvae exposed to corresponding quinpirole concentrations was observed (Fig. 3 c).

To evaluate the potential anxiety altering effect of quinpirole exposure, the time spent in, and the numbers of entries to, the outer ring area of the well (thigmotaxis) were evaluated. No significant difference between quinpirole treated and control larvae or between fed and non-fed larvae was observed in the time spent swimming in the outer ring area. However, a significant increase of frequency of entries to the outer ring was found in fed animals (ANOVA, Quinpirole concentration: $F_{(3, 184)} = 4.358$; $p = 0.0054$. Feed status: $F_{(1, 184)} = 36.98$; $p < 0.0001$. Interaction: $F_{(3, 184)} = 4.380$; $p = 0.0053$). Fed larvae exposed to 50.0 μM ($p = 0.0135$) quinpirole presented increased number of entries to the outer ring area of the well when compared to fed controls, and when compared to non-fed larvae exposed to 50.0 μM ($p < 0.0001$) (Fig. 3 d, e).

Moreover, for in both fed and non-fed groups, quinpirole treated larvae exhibited increased absolute turn angle, while feed status and the interaction between these two factors were non-significant (ANOVA, Quinpirole concentration: $F_{(3, 160)} = 22.77$; $p < 0.0001$. Feed status: $F_{(1, 160)} = 0.3623$; $p = 0.5481$. Interaction: $F_{(3, 160)} = 0.2253$; $p = 0.8787$). Fed larvae exposed to 5.5 ($p = 0.003$), 16.7 ($p = 0.0024$) and 50.0 μM ($p = 0.0001$) exhibited increased turn angle when compared to fed controls. Non-fed larvae exposed to 5.5 ($p = 0.0015$), 16.7 ($p < 0.0001$) and 50.0 μM ($p < 0.0001$) also presented significant increase in turn angle when compared to non-fed controls (Fig. 3 f).

Additionally, fed larvae exposed to quinpirole exhibited a unique alteration in their swim path pattern. During the one-hour exposure, an increased percentage of these larvae (25%, 66.7%, and 70.8% of the animals exposed to 5.5, 16.7, and 50.0 μM, respectively) exhibited fast and repetitive circular swimming interrupted by bouts of immobility (ANOVA, Quinpirole concentration: $F_{(3, 184)} = 8.185$; $p < 0.0001$. Feed status: $F_{(1, 184)} = 21.75$; $p < 0.0001$. Interaction: $F_{(3, 184)} = 8.185$; $p < 0.0001$). Importantly, this behavior appeared to be quinpirole concentration-dependent. However, the significant effect was observed only in fed animals treated with quinpirole at 50.0 μM when compared to fed controls ($p < 0.0001$) and non-fed animals exposed to 50.0 μM ($p < 0.0001$) (Fig. 3 g). Temporal variation over the 1 h exposure is shown in the supplementary material (Supp Figs. 1, 2).

3.2.1. Fluoxetine exposure

To reverse the quinpirole induced behavior changes (anxiogenic effects), larvae were exposed to fluoxetine (1 mg/L). Four experimental groups were tested additionally to the control group. After 1 h exposure to the highest quinpirole concentration (50.0 μM), larvae were exposed for an additional hour to the following treatments: water, 50.0 μM Quinpirole, 1.0 mg/L Fluoxetine, and 50.0 μM Quinpirole +1.0 mg/L Fluoxetine. The behavior of larvae was monitored during both the first 1 h quinpirole (or water) exposure period and the subsequent 1 h exposure period.

As observed in the first experiment described above, larvae exposed to quinpirole for one hour appeared to exhibit the fast and repetitive circular swim pattern (Fig. 4). However, ANOVA showed that the frequency of circling did not significantly change in any group between the first and second hour of testing/treatment (Treatment: $F_{(4, 114)} = 1.853$, $p = 0,1236$; Exposure period: $F_{(1, 114)} = 5.521$, $p = 0.0205$; Interaction: $F_{(4, 114)} = 1.607$, $p = 0.1773$). Temporal variation over the hour 1 and 2 is shown in the supplementary material (Supp Fig. 3).

3.3. Optomotor behavior

Two different optomotor tasks were performed at the end of quinpirole treatment. First, the larvae's capacity to respond to a non-aversive visual stimulus was tested (Fig. 5 a). Larvae of fed and non-fed groups did not appear to differ in either task. However, quinpirole treatment appeared to reduce the larvae's capacity to respond to (follow) the black-white moving stripes compared to control larvae. It appeared that among the fed larvae all quinpirole concentrations were able to impair this response, while among the non-fed larvae only the two highest quinpirole concentrations seemed to be effective (ANOVA, Quinpirole concentration: $F_{(3, 136)} = 38.10$; $p < 0,0001$. Feed status: $F_{(1, 136)} = 5.450$; $p = 0.0210$. Interaction: $F_{(3, 136)} = 0.4982$; $p = 0.6841$). Fed larvae exposed to 5.5 ($p = 0.0116$), 16.7 ($p = 0.0003$) and 50.0 μM ($p < 0.0001$) were impaired at following the movement of black-white stripes compared to fed controls. Non-fed animals exposed to 16.7 ($p = 0.0051$) and 50.0 μM ($p < 0.0001$) were also impaired compared to non-fed controls (Fig. 5 a).

In addition, we also evaluated the larvae's ability to escape from an aversive visual stimulus. After quinpirole exposure, non-fed animals appeared to show impairment in escaping the red "bouncing ball" when compared to the respective controls and fed animals (ANOVA, Quinpirole concentration: $F_{(3, 256)} = 7.233$; $p = 0.0001$. Feed status: $F_{(1, 256)} = 18.81$; $p < 0.0001$. Interaction: $F_{(3, 256)} = 1.677$; $p = 0.1723$). Fed larvae exposed to 16.7 μM ($p = 0.0376$) presented an impairment escaping the aversive stimulus when compared to fed control. Non-fed larvae exposed to 5.5 ($p = 0.0082$) and 50.0 μM ($p = 0.0181$) also presented an impairment to escape when compared to non-fed control. Furthermore, comparing fed and non-fed animals we observed that fed larvae exposed to 5.5 ($p = 0.0140$) and 50.0 μM ($p = 0.0473$) showed a better response than non-fed animals exposed to the same quinpirole concentrations when escaping the aversive stimulus (Fig. 5 b).

4. Discussion

In the present study, we analyzed the effects of quinpirole exposure on the behavior of zebrafish larvae and also tested whether these effects may be influenced by feeding status (testing fed versus non-fed larvae). We found significant interaction between quinpirole treatment and feeding status in several behaviors. However, we did not detect any gross morphology alterations and/or effects on heartbeat. The

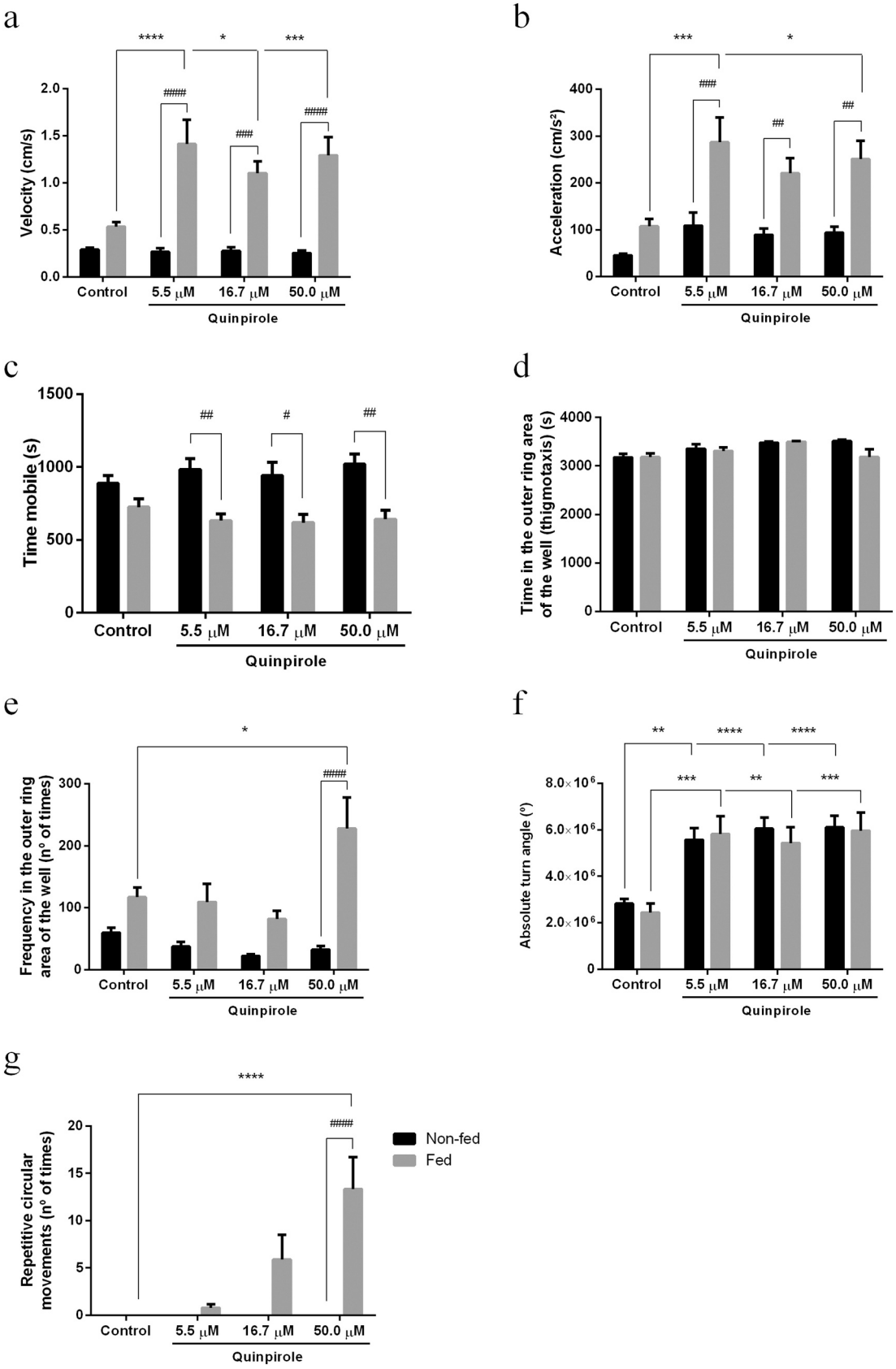

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Fig. 3. Exploratory behavior of zebrafish larvae during 1-h exposure of quinpirole. Mean ± S.E.M are shown. Sample sizes are $n = 24$ for each group. Velocity (a), acceleration (b), time mobile (c), time in the outer ring area of the well (d), frequency in the outer ring area of the well (e), absolute turn angle (f) and repetitive circling movements (g) were analyzed during the one-hour long test. Black bars indicate non-fed group and grey bars indicate fed group. Two-way ANOVA was used, followed by post-hoc Tukey's HSD test. Comparisons between control and the quinpirole concentrations groups, as well as between fed or non-fed groups, are indicated by asterisk. * indicates significant difference at $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$. Comparisons between fed and non-fed group exposed to the same quinpirole treatment (water or quinpirole concentrations) are indicated by hash. # represents significant difference at $p \leq 0.05$, ## $p \leq 0.01$, ### $p \leq 0.001$ and #### $p \leq 0.0001$. Note the robust and significant quinpirole induced changes in fed but not in non-fed larvae in some behaviors (Velocity (a), Acceleration (b), Frequency of entries to the outer ring (e) and Repetitive circling (g)), and also the robust feed status independent quinpirole effect in another behavior, Absolute turn angle (f). Also note the feed status dependent effect in quinpirole treated larvae in Time spent mobile (c). For details of results of statistical analysis, see Results.

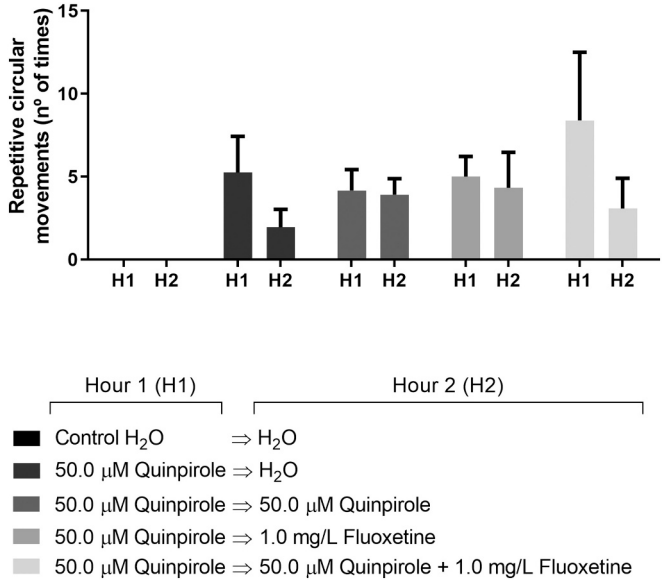

Fig. 4. Frequency of repetitive circling movements exhibited during the first and second hour of treatment. Mean ± S.E.M are shown. Sample sizes are n = 24 for each group. The experimental design, i.e. the drug treatments employed for the first hour (H1) and the second hour (H2) are shown below the bar graph with different shading corresponding to the different treatment groups indicated. Two-way repeated measure ANOVA was used. For detailed results of statistical analyses, see Results.

significant interaction between quinpirole treatment and feed status was manifested, for example, in changes in velocity and acceleration. These measures of activity were both increased by quinpirole but only in fed and not in non-fed larvae. Similarly, the time spent mobile significantly differed between fed and non-fed larvae across the three quinpirole exposed groups but not for the control groups. Last, the number of larvae showing repetitive circling behavior was dose dependently and dramatically increased by quinpirole treatment, but only in fed larvae. On the other hand, turn angle appeared to be increased by quinpirole treatment irrespective of the feed status of the larvae, and time spent in the outer ring (thigmotaxis) was not affected by quinpirole treatment or by feed status.

The significant interaction between the effects of quinpirole and feed status we report here in zebrafish for the first time is in good agreement with findings obtained in mammals. For example, different diets have been found to lead to several changes in processes involving the dopaminergic system (Sevak et al., 2008; Baladi and France, 2009; Briguglio et al., 2018). In rodents, limiting food intake increased sensitivity to the behavioral effects of dopamine receptor agonists. Furthermore, short-term access to high fat diet has been found to increase and long-term access to decrease dopamine D2 receptor binding (Huang et al., 2006; South and Huang, 2008). Moreover, food restriction increased dopamine D2 receptor binding and coupling between dopamine receptors and G proteins, and decreased dopamine transporter activity in mammals (Patterson et al., 1998; Carr, 2002; Thanos et al., 2008).

During the quinpirole exposure in novelty exploration task, we also observed behavioral changes induced by quinpirole in two subsequent tasks that have been designed to test responses to visual stimuli, the optomotor task and an aversive stimulus avoidance task. Optomotor tasks are used to assess the reflexive response of larvae to moving visual stimuli. Other visual tasks, e.g. the aversive stimulus presentation-based task we used here, evaluate anxiety-like responses and may measure escape-behavior as it would be performed by the fish in response to the presence of natural threats, e.g. predators (Colwill and Creton, 2011; Pelkowski et al., 2011; Nery et al., 2014). In the present study, we found quinpirole to impair the optomotor response. The impairment appeared dose dependent but independent of feed status. However, avoidance of

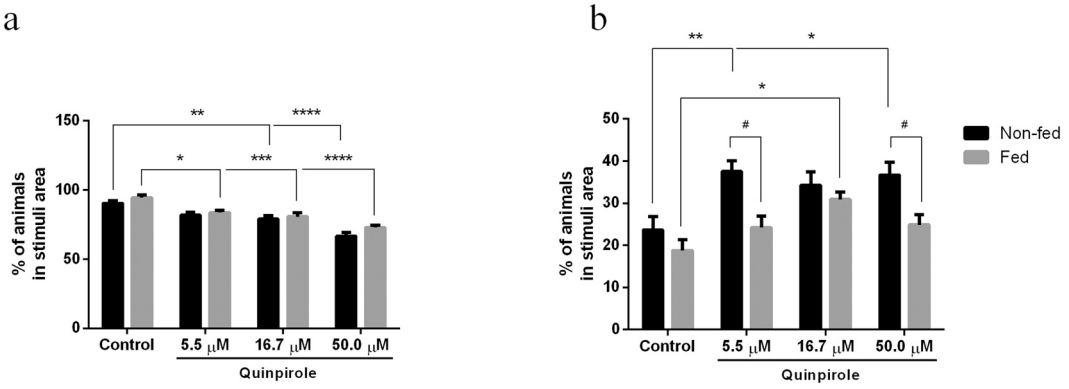

Fig. 5. Visual stimulus induced behaviors. Response to moving stripes (optomotor response) (a) and avoidance of a moving aversive visual stimulus (b) were evaluated at the end of 1 h of quinpirole exposure. Mean ± S.E.M are shown. Sample sizes are n = 30 for each group. Black bars indicate non-fed group and grey bars indicate fed group. Two-way ANOVA was used, followed by post-hoc Tukey's HSD test. Comparisons between control and quinpirole concentrations of each group, fed or non-fed, are indicated by asterisk. * represents significant difference at $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$. Comparisons between fed and non-fed group exposed to the same treatment (water or quinpirole concentrations) are indicated by hash. # represents significant difference at $p \leq 0.05$. For detailed results of statistical analyses, see Results.

an aversive visual stimulus, the "bouncing red ball", we found to depend upon both quinpirole treatment and feed status. Controls and fed larvae exposed to quinpirole showed strong avoidance of the aversive stimulus, i.e. did not choose the side where the stimulus was present. However, the quinpirole (5.5 and 50.0 μM) treated non-fed larvae tended to ignore this stimulus and chose the stimulus side in higher numbers. These results are noteworthy as they imply that the effect of quinpirole on the simple optomotor reflex, and thus visual processing per se, may not have been influenced by feed status. However, when vision was used by the experimental zebrafish for a more cognitively demanding task, i.e. risk assessment; feed status did make a difference. These results are in agreement with a large body of literature all showing that well-fed prey fish, unlike hungry ones, tend to take less risks and tend not to investigate predators or aversive cues (Lönnstedt et al., 2012; Filosa et al., 2016).

Dopaminergic signaling is known to regulate several behavioral processes and a wide array of cerebral functions related to visual stimulus processing and cognition (Goldman-Rakic, 1998; Bjorklund and Dunnett, 2007). Quinpirole exposure is also known to alter cognitive processes in a variety of species (Thacker et al., 2006; Stuchlik et al., 2007; Herold, 2010). For example, enhanced performance of zebrafish was observed in a plus-maze associative learning paradigm when quinpirole was administered immediately before training and probe test, but not when it was administered after (Naderi et al., 2016a). The same authors, using a complex maze to assess cognitive performance, observed that pre- and post-training exposure to quinpirole significantly impaired learning and memory in zebrafish (Naderi et al., 2016b). Thus, we conclude that the feed status dependent quinpirole effects are likely due to the involvement of the dopaminergic system in cognitive function and thus mechanistically may be explained by the interaction between this neurotransmitter system with several others.

Dopamine, similarly to its function in mammals, also regulates locomotor activity in zebrafish and, for example, is required for the initiation of movement (Thirumalai and Cline, 2008; Souza et al., 2011; Lambert et al., 2012; Irons et al., 2013; Ek et al., 2016). In the present study, during quinpirole exposure, we observed increased locomotor activity at all concentrations tested (5.5, 16.7, and 50.0 μM). The effect of increased locomotor activity observed in the present study is partially in agreement with data from other studies, in which low and intermediate concentrations of quinpirole were found to increase activity. For example, Boehmler et al. (2007), using similar concentrations (12.5 μM for 60 min) and length of exposure to what we employed in our study, observed hyperactivity in zebrafish larvae, a finding also consistent with what was previously reported by Irons et al. (2013). In the latter study, the authors reported increased larval locomotion in a light/dark task. In the dark, quinpirole increased activity at 16.7 μM. In the light, increased activity was seen at 5.5 and 16.7 μM quinpirole concentrations. In contrast, two other studies found decreased movements and activity after quinpirole exposure (Souza et al., 2011; Lange et al., 2018), contradicting findings that may be due to different procedures and/or different genetic background of fish used. One important factor in the procedures employed in these prior studies may have been the feeding status of the larvae tested. However, previous studies that evaluated quinpirole exposure effects in zebrafish larvae did not describe the feeding status of their subjects.

Given that zebrafish larvae are usually started to be fed from 7 dpf (Westerfield, 2007), larvae of these previous studies were likely unfed prior to quinpirole exposure (Boehmler et al., 2007; Irons et al., 2013; Souza et al., 2011; Lange et al., 2018). Notably, zebrafish larvae start hunting for food by 5 dpf, and yolk-sac reserves and exogenous food sources are consumed concurrently (Trotter et al., 2009). Also, by this age, dopamine receptors are already functional in this species (Boehmler et al., 2004, 2007; Li et al., 2007). Thus, lack of feeding through 7 dpf as employed in the above cited prior studies likely did affect the dopaminergic system and thus also the effects of quinpirole treatment.

In addition to increased locomotor activity, phenotypes related to anxiety-like behavior were observed in fed animals exposed to quinpirole, such as thigmotaxis, erratic and repetitive behavior, and absolute turn angle. To date, there are no studies describing these effects in zebrafish larvae in response to quinpirole exposure in the context of nutritional state. Although mechanisms affecting anxiety levels are numerous, anxiety has been found to depend also upon energy states. Similarly to what we observed here, studies with rodents have also shown that hunger decreased while feeding increased anxiety-like behaviors (Inoue et al., 2004; Levay et al., 2007; Burnett et al., 2016; Li et al., 2019).

The behavioral effects observed in the present study suggesting hyperactivity, hyper-reactivity, elevated anxiety, and increased repetitive behavior are parts of the behavioral spectrum observed in mammalian laboratory animal models of human neuropsychiatric disorders, such as schizophrenia, obsessive-compulsive disorders and anxiety (Brown et al., 2012; Parker et al., 2013; D'Angelo et al., 2014; Archer and Kostrzewa, 2016; Stuchlik et al., 2016; Bortolato and Pittenger, 2017; Szechtman et al., 2017; Demin et al., 2019). Zebrafish models of these disorders, except for anxiety (Kalueff et al., 2013; Khan et al., 2017), are lacking. Nevertheless, the zebrafish has been suggested as a possibly appropriate animal model to study obsessive-compulsive disorder with expected endophenotypes, as anxiety-like behavior, impulsivity, compulsivity, and stereotypic movements (D'Amico et al., 2015; Meshalkina et al., 2017; Parker, 2017; Zabegalov et al., 2019).

Fluoxetine, a selective serotonin reuptake inhibitor, has been found to be efficacious in zebrafish in prior studies (Maximino et al., 2011; Pittman and Hylton, 2015; Giacomini et al., 2016). Fluoxetine is traditionally used to treat depression as well as obsessive-compulsive disorder symptoms in humans and is also considered an important tool for validating animal models of these disorders (Zohar et al., 2000; D'Amico et al., 2015). In the current study, we found fluoxetine exposure not attenuating repetitive movement induced by quinpirole in fed larvae. For the best of our knowledge, the use of fluoxetine to reverse or attenuate quinpirole exposure effects has not been demonstrated before in zebrafish. In rodents, this effect has been studied, and fluoxetine has been used to validate rodent models of neurological disorders. However, the results have been contradictory. Exposure to fluoxetine alone or in combination with other drugs has been found to revert quinpirole effects, by decreasing locomotor activity and stereotypic behavior (Korff et al., 2008; Rogóz and Skuza, 2009; Sanikhani et al., 2020). On the other hand, fluoxetine exposure has also been observed to enhance effects of quinpirole (Collu et al., 1997; Ainsworth et al., 1998; Marsteller et al., 2009). Thus, the question whether fluoxetine can reverse or attenuate the effects of quinpirole exposure needs to be further studied in zebrafish. Different concentrations, exposure period, association with other drugs, and the study of targets other than those associated with the serotonergic system may be interesting. Nevertheless, considering we observed several behavioral alterations in the zebrafish related to phenotypes seen in human neurological disorders, we propose that quinpirole exposure may be a good start for modeling neurological disorders, particularly obsessive-compulsive disorder, in the zebrafish, a simple vertebrate species.

The next question we consider is the possible mechanisms underlying quinpirole's behavioral effects. Quinpirole is a D2/D3 dopamine receptor agonist which has a characteristic dose-dependent biphasic effect profile, reducing motor activity at low and increasing motor activity at high concentrations in mammals (Li et al., 2010). Such biphasic response is believed to result from the dose dependent activation of D2/D3 receptors, owing to the fact that the D2 receptor is expressed both pre- and post-synaptically, whereas D3 exclusively post-synaptically in numerous mammalian brain areas (De Mei et al., 2009). In mammals, low concentrations of D2 receptor agonists primarily activate presynaptically expressed D2 receptors, which act as auto-receptors providing a negative feedback loop for the dopaminergic neuron. Thus, activation of presynaptic D2-receptor leads to reduction of dopamine release/synthesis, which in turn decreases locomotion. At high concentrations,

D2 receptor agonists act through the post-synaptic D2 receptor and induce hyperactivity (Van der Weide et al., 1988; Beninger et al., 1991; Missale et al., 1998; Beaulieu and Gainetdinov, 2011). In zebrafish, the dopamine D2 and D3 receptors are pre- and post-synaptically expressed. Initially, i.e. in the first hours of development, the receptors are localized on postsynaptic membrane sites. However, as development progresses, i.e. by around 36 hpf, expression of receptors genes extends throughout the CNS and the microstructural localization changes from exclusively postsynaptic to both the pre- (auto-receptors) and the post-synaptic membrane of neurons (Boehmler et al., 2004).

The observed behavioral effects of quinpirole may also be due to a complex interplay between dose dependent pre- and post-synaptic D2-receptor activation as well as post-synaptic D3 receptor activation. For example, Tran et al. (2015) studying the behavioral effects of amisulpride, in zebrafish, found a biphasic dose-response in total distance traveled and in angular velocity (speed of turning). Amisulpride is a selective mammalian D2/D3 receptor antagonist, which at higher concentrations is known to bind post-synaptic D2/D3 receptors, whereas at lower concentrations it is selective for presynaptic mammalian D2 dopamine autoreceptors (Perrault et al., 1997; Schoemaker et al., 1997). Although the similarities are striking, unlike for mammals, such mechanistic details on the mode and location of action of quinpirole in zebrafish are lacking. Furthermore, the interaction between nutritional state and dopaminergic signaling in zebrafish has not been demonstrated before our current study. Thus, the question whether the mechanisms underlying this interaction in zebrafish are similar to those found in mammals also has not been addressed. Nevertheless, our current demonstration of this interaction now opens up a new research line in this direction.

In the present study, we investigated possible interactions between the feeding status and the effect of quinpirole exposure on the behavior of zebrafish. We found quinpirole exposure induced behavioral effects to be dependent upon feeding status of the zebrafish larvae. A limitation of our study, however, is that cellular and molecular analyses of the dopamine system were not performed. Furthermore, we cannot exclude that part of the observed effects in fed group could be due to the fact that the fed larvae were on an environment with greater energy availability and, therefore, had an increase in swimming activity, which was amplified after quinpirole exposure. However, we emphasize that in none of the evaluated behavioral tasks, fed and non-fed larvae in the control group showed a significant difference. Also, in the parameters related to activity, we found no difference between non-fed control and non-fed quinpirole-treated larvae, indicating that the effects are resulting from an interaction between quinpirole exposure and feeding status. Furthermore, we acknowledge that numerous additional behavioral tests, perhaps more specifically designed in neuropsychiatric conditions in mind, may need to be performed to establish and validate the quinpirole-zebrafish as a translationally relevant model.

In summary, our study demonstrated, for the first time, that nutritional status affects behavioral effects of quinpirole exposure in zebrafish. Quinpirole effects highly observable specifically in fed zebrafish larvae included increased activity, anxiety-like behavior and repetitive movements, responses analogous to those seen in some human neurological disorders and their mammalian models. Importantly, no behavioral differences were observed between fed and non-fed controls in any tests performed, suggesting that the observed effects are not due to additive effects of feeding or/and quinpirole exposure, but rather to the interaction between these factors. For the best of our knowledge, such interaction has not been demonstrated before in zebrafish. These results are noteworthy both from practical as well as empirical standpoints.

5. Conclusion

Our study demonstrated that feeding status alters exploratory and anxiety-like behaviors in zebrafish larvae exposed to quinpirole. These findings highlight the importance of controlling nutritional status in the analysis of the dopaminergic system in psychopharmacology as well as behavioral genetic studies of zebrafish. They also open a new research avenue to the mechanistic analysis of the dopaminergic system in neuropsychiatric disorders using this simple vertebrate.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - finance code 001, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Proc. 420695/2018–4), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS; 17/2551–0000977-0) and Instituto Nacional de Ciências e Tecnologia para Doenças Cerebrais, Excitotoxicidade e Neuroproteção. C.D.B. (Proc. 304450/2019–7) was the recipients of a fellowship from CNPq. RG is supported by NSERC Canada (311637) and the University of Toronto Distinguished Professorship Award.

Ethical statement

All protocols were approved by the Institutional Animal Care Committee from Pontifícia Universidade Católica do Rio Grande do Sul (CEUA-PUCRS, permit number 8854) and Comply with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA). This study was registered in the Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado - SISGEN (Protocol No. A3B073D).

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2020.110179.

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