Locomotor Behavior and Memory Dysfunction Induced by 3-Nitropropionic Acid in Adult Zebrafish: Modulation of Dopaminergic Signaling

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Abstract

Huntington's disease (HD) is a progressive neurodegenerative disease characterized by neuropsychiatric disturbance, cognitive impairment, and locomotor dysfunction. In the early stage (chorea) of HD, expression of dopamine D_2 receptors (D_2R) is reduced, whereas dopamine (DA) levels are increased. Contrary, in the late stage (bradykinesia), DA levels and the expression of D_2R and dopamine D_1 receptors (D_1R) are reduced. 3-Nitropropionic acid (3-NPA) is a toxin that may replicate HD behavioral phenotypes and biochemical aspects. This study assessed the neurotransmitter levels, dopamine receptor gene expression, and the effect of acute exposure to quinpirole (D_2R agonist) and eticlopride (D_2R antagonist) in an HD model induced by 3-NPA in adult zebrafish. Quinpirole and eticlopride were acutely applied by i.p. injection in adult zebrafish after chronic treatment of 3-NPA (60 mg/kg). 3-NPA treatment caused a reduction in DA, glutamate, and serotonin levels. Quinpirole reversed the bradykinesia and memory loss induced by 3-NPA. Together, these data showed that 3-NPA acts on the dopaminergic system and causes biochemical alterations similar to late-stage HD. These data reinforce the hypothesis that DA levels are linked with locomotor and memory deficits. Thus, these findings may suggest that the use of DA agonists could be a pharmacological strategy to improve the bradykinesia and memory deficits in the late-stage HD.

Keywords Dopaminergic system · Huntington's disease · Locomotor dysfunction · Memory loss · 3-Nitropropionic acid · Zebrafish

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Introduction

Huntington's disease (HD) is a devastating and incurable heritable neurodegenerative disease caused by an increase in the number of repeats of cytosine-adenine-guanine (≥ 36 repeats) trinucleotide in exon 1 of the huntingtin (*Htt*) gene, which leads to an expanded polyglutamine (poly Q), resulting in expression of mutant *Htt* protein [1–3].

The neuropathological mark of HD is a notable neuronal loss in the striatum (putamen and caudate nucleus). As the disease progresses, other brain regions, such as the cerebral cortex, substantia nigra, globus pallidus, subthalamic nucleus, thalamus, and hypothalamus, are damaged [4]. The corticostriatal pathways involved in the control of many behaviors are injured in HD. This leads to an imbalance in several neurotransmitters, including dopamine (DA), serotonin (5-HT), gamma-aminobutyric acid (GABA), and glutamate (GLU), that constitute the corticostriatal pathways and consequently reflect in the symptoms of disease [5].

Clinically, HD is characterized by a neurobehavioral progressive triad of symptoms, which include locomotor dysfunction, neuropsychiatric disturbance, and cognitive impairment [6]. Locomotor dysfunction is divided into two stages (early and late): in the early stage, the neurons of the indirect striatum pathway (its action inhibits voluntary movements) degenerate and produce involuntary movements, known as chorea. In the late stage, there is also the degeneration of direct pathway neurons (acts by initiating voluntary movements), a situation in which voluntary movements are impaired, causing bradykinesia, rigidity, and locomotor incoordination [7].

The DA modulates several physiological functions, including locomotor control, memory, motivation, reward, and pleasure. DA neurons extend from the substantia nigra pars compacta to the striatum originating the nigrostriatal pathway [8]. DA is synthesized from tyrosine that, by the action of tyrosine hydroxylase, is converted to levodopa, which then is decarboxylated to DA [8]. After, DA is stored in presynaptic vesicles, and then it is transported by vesicular monoamine transporter (VMAT2), into synaptic vesicles, where it is stored until released in the synaptic cleft [8]. After released, DA levels are modulated by two distinct phases: phasic, which occurs explosive firings of neurons resulting in a fast and transient increase of DA concentrations near to presynaptic terminal; and the tonic phase occurs when DA is spontaneously released by neurons and is regulated by the activity of other neurons and neurotransmitter reuptake [8]. Finally, DA acts via two major families of receptors: D_1-like receptors, which include D_1 (D_1R), and D_5 (D_5R), which are facilitatory and involved in postsynaptic DA mediated effects; whereas D_2-like receptors are inhibitory, comprise D_2 (D_2R), D_3 (D_3R), and D_4 (D_4R) receptors, localized in post- and presynaptic DA neurons [8].

The nigrostriatal tract plays a key role in locomotor control, and at the same time, this pathway has an essential role in HD pathophysiology. Biphasic changes in DA levels are found in either HD subjects or animals. Consistent with this, in the early stage of the disease occurs an increase in DA levels in subjects with the disease [9, 10] and HD animal models [11], while in post-mortem analysis of patients [9, 10] and animal models [11] with late stage of HD, the DA levels are reduced [9–11]. Moreover, in both HD subjects and animal models have been demonstrated a decrease in D_2R in the early stage and then a decrease in D_1R in the late stage of the disease [12, 13].

One of the most used animal models to understand HD mechanisms is the 3-nitropropionic acid (3-NPA) pharmacological model. 3-NPA is a neurotoxin that causes irreversible inhibition of complex II of the respiratory chain [14], producing biochemical changes (ATP decrease levels, oxidative stress, neuronal death), such as perceived in the autopsy cerebrum of HD patients [15–18]. Animals treated with 3-NPA also display neurobehavioral phenotypes as detected in patients with advanced (late-stage) HD [19, 20].

In this way, the zebrafish is a suitable animal model for studying diverse neurodegenerative diseases [20–22] due to its high genetic and physiological similarities with mammals, responses highly sensitive to pharmacological modulations, and myriad advantages over other animal models [23]. A previous study of our research group demonstrated that the 3-NPA chronic treatment in adult zebrafish caused hypolocomotion and memory impairment in animals that mimics the advanced (late-stage) HD symptoms [20]. Furthermore, previous studies have described dopamine receptor subtypes D_1R (*drd1a*, *drd1b*) and D_2R (*drd2a*, *drd2b*) in zebrafish [24–27].

Overall mentioned above, it is important to point out that the pharmacological treatment for the late stage of HD is still scarce and that the dopaminergic system has an essential role in HD pathophysiology. Thus, we aimed to determine the alteration of brain DA levels and investigate the effects of dopamine receptor antagonist and agonist modulation in a pharmacological HD model induced by 3-NPA in adult zebrafish. We hypothesized that 3-NPA treatment would cause a reduction in DA levels and locomotor and memory phenotypes to those observed in the late stage of HD.

Material and Methods

Animals Maintenance

In this study, we used a total of 299 adult zebrafish (AB strain, 6–10 months, 0.3–0.4 g, male/female) obtained from our breeding colony. The animals tested were obtained from different mates from our animal facility and were randomly distributed to the experimental groups. Previous to the treatment, the fish were kept in recirculating systems (Zebtec, Tecniplast, Italy) containing reverse osmosis water, equilibrated to reach the species standard temperature (28 °C ± 2 °C), pH (7.0 to 7.5), conductivity (300–700 μS), hardness (80–300 mg/L), ammonia (< 0.02 mg/L), nitrite (< 1 mg/L), nitrate (< 50 mg/L), and chloride levels (0 mg/L). Animals were exposed to a light/dark cycle of 14/10 h, respectively. All animals were fed with paramecium between 6- and 14-day post-fertilization (dpf) and after 14 dpf with commercial flakes (TetraMin Tropical Flake Fish®) three times per day that were supplemented with brine shrimp [28]. All procedures for animal manipulations were according to the Brazilian Council of Animals Experimentation guidelines for Use of Fish in Research (CONCEA) and the Brazilian legislation (11.794/08). The sample number for behavioral analyses was reported by previous studies performed by our research group [20, 29] and other research groups that used zebrafish as an animal model [30, 31]. In addition,

the sample number for biochemical analyses was based on Altenhofen et al. [32] and Zanandrea et al. [33]. The protocols were approved by the Animal Care Committee of the Pontifical Catholic University of Rio Grande do Sul (CEUA-PUCRS, protocol number 9406/2019). This study was registered in the Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado-SISGEN (Protocol No. A3B073D).

Chemical Compounds

The drugs 3-NPA, quinpirole (QNP), eticlopride (ETI), tricaine, DA, 5-HT, and glutamic acid (GLU) were purchased from Sigma (St. Louis, MO, USA). 3-NPA, QNP, and ETI were dissolved in saline (0.9% NaCl). Tricaine was dissolved in the recirculating system water.

Experimental Procedures

In all experiments, the animals were distributed randomly into the experimental groups and kept in two different aquariums for each experimental group (2 aquariums per experimental group). The animals were treated with saline (vehicle, for the control group) and 3-NPA for 24 days (one injection every 4 days) and housed in these aquariums for 29 days. Each experimental condition was evaluated in fish obtained from 2 independent treatments. The animals from saline (vehicle, for the control group) and 3-NPA-treated groups were evaluated randomly in the behavioral assays.

Moreover, different fish were submitted to the locomotor activity test and the inhibitory avoidance task. The animals were not fed before all interventions. The fish were fed in the previous day before the tests at 12 p.m. and 5 p.m.

Treatments

3-NPA Treatment

The behavioral phenotype and biochemical alterations that mimics the HD were induced through the 3-NPA treatment in adult zebrafish. The animals were selected randomly and separated into two groups considering a density of three animals per liter: control and 3-NPA-treated groups. Fish were subjected to seven intraperitoneal (i.p.) injections of 3-NPA or saline (vehicle, for the control group) every 96 h, in a total of 24 days of treatment. Before the injection, the animals were anesthetized by immersion in 100 mg/L tricaine solution buffered with sodium bicarbonate. The animals received an i.p. injection at 60 mg/kg body weight 3-NPA or saline in a 10-µL volume. The dose was chosen according to a previous study performed by our group, which showed that the chronic treatment with 3-NPA in adult zebrafish at three doses, 10, 20, or 60 mg/kg, decreased the distance

traveled. The highest 3-NPA dose (60 mg/kg) had more robust neurobehavioral effects than 10 or 20 mg/kg [20]. Furthermore, the 3-NPA at 60 mg/kg promoted memory impairment, inducing phenotypes that closely resemble the behavioral symptoms of late-stage HD [20]. Also, we used only the saline (vehicle) as the control group because in previous experiments, there were no significant behavioral changes between the naïve group (without any manipulation) and the control (vehicle) group.

I.p. injections were administered using a 3/10-mL U-100 BD Ultra-Fine™ Short Insulin Syringe with 8 mm (5/16 in)×31 G short needle (Becton Dickinson and Company, New Jersey, USA). Subsequently, to anesthesia recovery, the animals were placed into their home aquarium. All groups (3-NPA and control animals) were maintained in the same essential parameters described above. During the treatment, there was a mortality of 25% for both groups (vehicle control and 3-NPA treatment). The experimental design is shown in Fig. 1a and Fig. 1b.

Dopaminergic Drugs on 3-NPA-Induced HD

To test the modulation of DA signaling on 3-NPA-induced HD in zebrafish, it was administered a single i.p. injection of quinpirole, eticlopride, or their respective vehicle, 30 min before the beginning of each behavioral experiment on day 29 (120 h) after the last injection of 3-NPA (day 25). QNP (5 mg/kg) and ETI (1 mg/kg) were injected via i.p. in a volume of 10 µL using a 3/10-mL U-100 BD Ultra-Fine™ Short Insulin Syringe with 8 mm (5/16 in)×31 G short needle (Becton Dickinson and Company, New Jersey, USA). Drug doses were chosen accordingly by Lukács et al. [34] and Taylor et al. [35], and the administration route was chosen following a previous study performed by our group [20]. Before drug or vehicle administration, animals were anesthetized by immersion in 100 mg/L tricaine solution buffered with sodium bicarbonate. Subsequently, the animals were placed in an aquarium with a recirculation system water to recover from anesthesia.

Biochemical Assays

Determination of Neurotransmitter Levels

To better characterize the HD model induced by 3-NPA in adult zebrafish described by Wiprich et al. [20], we assess the DA, 5-HT, and GLU levels in adult zebrafish whole brain on day 29 (120 h) after the last injection of 3-NPA (day 25). A total of 72 animals were used for the analysis. The samples were prepared based on Zanandrea et al. [33] with modifications. Briefly, each animal was cryoeuthanized, and six whole brains per group ($n=12$) were gently dissected with a scalpel blade and immediately placed in
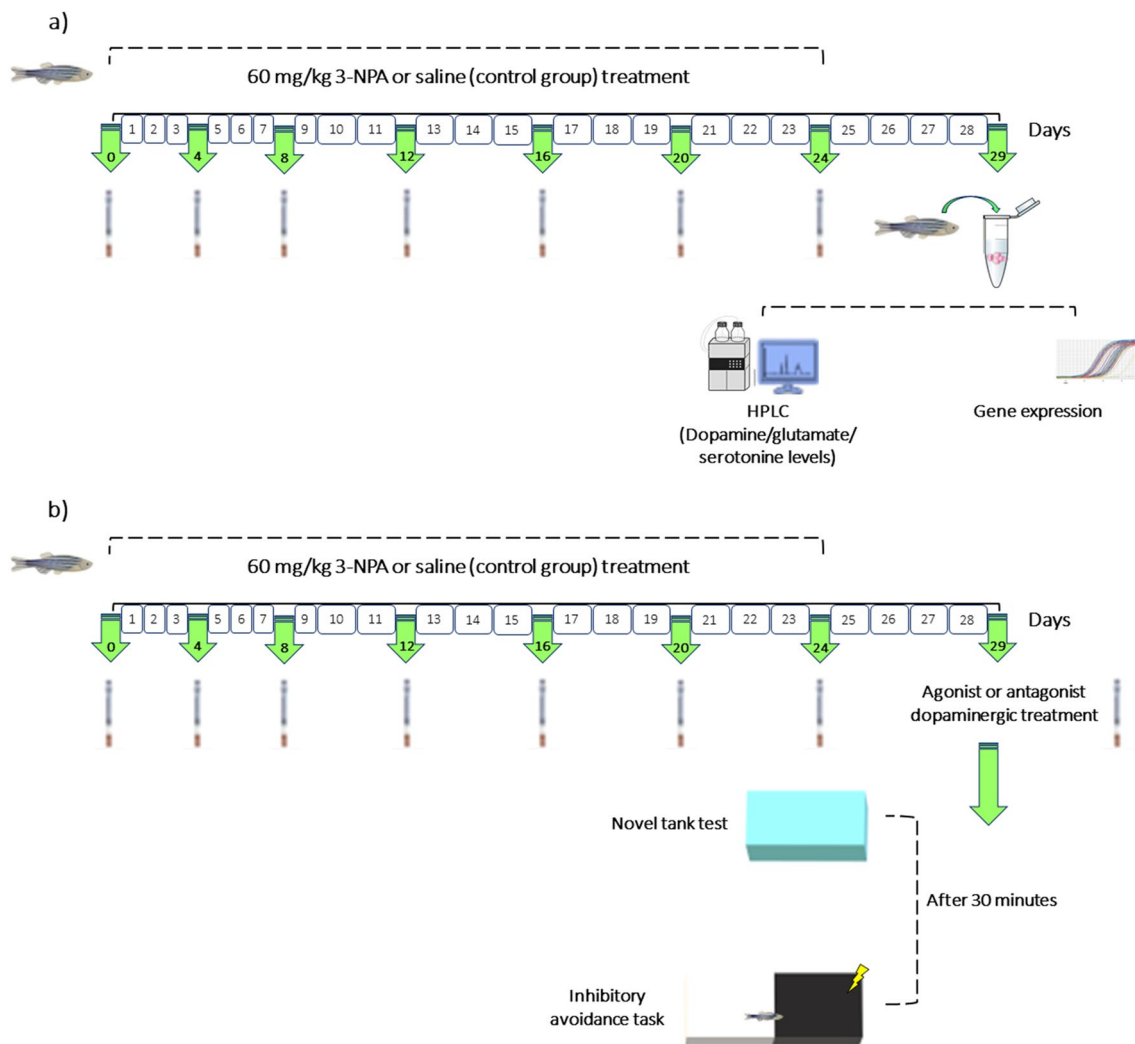
Fig. 1 Experimental design of 3-NPA treatment and **a** biochemical analysis and **b** behavioral assays performed in this study

an Eppendorf tube on ice. Then, the samples containing 300 µL of formic acid (0.1 M; Sigma-Aldrich, St. Louis, MO) were homogenized on ice with an Ultra-Turrax® homogenizer. Subsequently, the samples were centrifuged at 14,000 g for 20 min at 4 °C. The supernatant was transferred to a glass vial and injected into an Aquity I-Class UHPLC liquid chromatograph coupled to a Xevo TQ-S Micro system mass spectrometer (LC–MS/MS, Waters, Milford, MA, USA). The chromatographic separations were performed on a column Zorbax Eclipse Plus C18 RRHD (5×2.1 mm, 1.8 µm, Agilent, Paolo Alto, USA) using a mobile phase containing (A) 0.1% formic acid and (B) acetonitrile with 0.1% formic acid in gradient mode. The gradient started with 2% of B and next was set to 95% of B after 4.5 min remaining in this mode for 1.3 min before returning to the start condition. The total time of the chromatographic run was 7.5 min. The mobile phase flow was 0.2 mL/min with a column temperature of 40 °C, and

the injected extract volume was 10 µL. The spectrometer was operated in MRM mode, and the analytes were measured with the following transition (m/z): DA, 154.1/137.1 for quantification and 154.1/119.1 for confirmation; GLU, 148.0/84.0 for quantification and 148.0/102.2 for confirmation; 5-HT, 177.0/160.0 for quantification and 177.0/115.0 for confirmation. Quantifications were executed by external standardization, and the calibration curves were constructed with the concentrations of 1.0, 5.0, 10.0, 15.0, and 20.0 ng/mL for DA and 5-HT, and GLU at 0.1, 0.5, 1.0, 1.5, and 2.0 µg/mL.

The final results were adjusted by the protein concentration of the samples. The protein content was determined by Coomassie blue method [36], and for the calibration curve was used the bovine serum albumin as the standard.

Considering that brain neurotransmitter content of the pool can be affected by factors such as brain dimensions

and the contamination of water during its collection, our group has normalized the concentration of neurotransmitters in zebrafish brain by the protein content of the homogenate and the results have been presented [33], as nanogram of neurotransmitter per mg of the protein content of the pool.

Gene Expression Determination by RT-qPCR

To understand the molecular mechanisms involved in the HD model induced by 3-NPA in adult zebrafish, we analyzed the dopamine receptor gene expression by the real-time quantitative polymerase chain reaction (RT-qPCR) technique. Each zebrafish ($n = 12$) was euthanized by hypothermal shock. Subsequently, each animal's whole brain (five per group) was dissected with a scalpel blade, placed in an Eppendorf, and frozen in liquid nitrogen. After, we added TRIzol® Reagent and the samples were maintained at -80 °C. We analyzed genes of four dopamine receptor subtypes (*drd1a*, *drd1b*, *drd2a*, *drd2b*). The molecular analysis was performed according to Wiprich et al. [20]. Briefly, the total RNA was isolated with TRIzol® Reagent (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and their purity and concentration were determined with a NanoDrop Lite (Thermo Fisher Scientific, Waltham, Massachusettes, USA). Then, the RNA was treated, and the cDNA was synthesized. RT-qPCR was evaluated using SYBR® Green I (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to detect double-strand cDNA synthesis on the 7500 Real-time PCR System (Applied Biosystems, Foster City, California, USA). All real-time assays were carried out in quadruplicate. The genes used as a reference for normalization were ef1a and rpl13a. The efficiency per sample was calculated using LinRegPCR version 2021.1 Software (http://LinRegPCR.nl), and the stability of the references genes and the optimal number of reference genes according to the pairwise variation (V) were analyzed by GeNorm 3.5 Software (http://medgen.ugent.be/genorm/). Relative mRNA expression levels were determined using the $2^{-\Delta\Delta Cq}$ method [37, 38]. The primer sequences are shown in Table 1.

Behavioral Analysis

Locomotor Activity Test

The experiment was performed as described previously by our group according to Wiprich et al. [20]. All the groups were tested on two different experimental days. The test was performed between 8:30 a.m. and 12:00 p.m. The total number of animals used was 92 ($n = 12$ per group). Each animal was placed individually in test aquariums (30 cm $\times$ 15 cm $\times$ 10 cm; L $\times$ H $\times$ W) containing water obtained from the recirculating system (Zebtec system). After 60 s habituation, their locomotor behavior was recorded for 5 min [20]. Later, the records were analyzed with the EthoVision® XT tracking software (version 11.5, Noldus) at a rate of 30 frames per second. The parameters evaluated were distance traveled (m) and velocity (m/s, the ratio between distance traveled and movement). The parameter movement was considered the mobile time when the velocity was higher than 0.6 cm/s. When the animal reached the stop velocity (less than 0.59 cm/s), it was considered immobile time [20].

Inhibitory Avoidance Task

A total of 75 animals were used. We conducted the test ($n = 10$ to 11 per group) between 8:30 a.m. and 12:00 p.m. [20, 39]. We evaluated the inhibitory avoidance task in two sessions denominated training and test when between the sessions, there was an interval of 24 h. Individually, in both sessions, the animals were placed in an experimental aquarium with recirculating system water in the white side (left) with the guillotine door closed for 1 min to acclimatize animals. After this period, the guillotine door was lifted. In the training session, when the animal crossed into the black side (right), the guillotine door was closed, and the animal received a 3 ± 0.2 V AC electric shock pulsed for 5 s through two electrodes. The animal was gently removed from the apparatus and placed in its housing tank with only recirculating system water until the test session. The test session repeated the same protocol as the training session, except for not applying the electric shock. Further, the water in the test tank (apparatus) was

Table 1 Primer sequences used for each gene

Gene	Forward primer	Reverse primer	Reference
ef1a	5'CTGGAGGCCAGCTCAAACAT-3'	5'ATCAAGAAGAGTAGTACCGCTAGCATTAC-3'	Tang et al., 2007 [38]
rpl13a	5'TCTGGAGGACTGTAAGAGGTATGC-3'	5'AGACGCACAATCTTGAGAGCAG-3'	Tang et al., 2007 [38]
drd1a	5'-CTCAAATGTGGACTCGGAAAG-3'	5'-GAGAGAAGAGTTAGCCCATCCA-3'	Paiva et al., 2020 [27]
drd1b	5'-GACGGTGAACAAACTGCTGA-3'	5'-CTTACACGTGAATCGGAGCA-3'	Lv et al., 2019 [25]
drd2a	5'-AGTGCCGTAAACCCAATC-3'	5'-GTATCATTTCCATCCCTTTCTG-3'	Lv et al., 2019 [25]
drd2b	5'-TCTCCGTTGTTTGGGTGCTT-3'	5'-CCATCCCGTGTAGCTGTGTT-3'	Thörnqvist et al., 2020 [26]

changed after three animals tested to avoid the influence of alarm substance in the test. The latency to enter the black compartment during each session was measured to evaluate memory retention. A 180-s ceiling was imposed on test session latency measurements. See more details in Wiprich et al. [20] and Blank et al. [39].

Statistical Analysis

To assess if the data were normally distributed, we applied the Shapiro–Wilk test for all experiments. Normal data from locomotor activity test data were evaluated by two-way ANOVA followed by Bonferroni as a post hoc test. The non-normal data for the locomotor activity test were adjusted through the log-transformation and analyzed using two-way ANOVA. Non-parametric tests analyzed data from the inhibitory avoidance task. The training and test latencies for each group were evaluated using the Mann–Whitney U matched pairs test, while the latencies of multiple groups were analyzed using Kruskal–Wallis and Mann–Whitney U tests. Also, data from neurotransmitter levels were determined by Student's t-test, while the Mann–Whitney test for unpaired samples analyzed gene expression data.

The exclusion criteria were based as follows: locomotor activity test, the fish were excluded when the software loose more than 10% of the total time spent in the arena; for the inhibitory avoidance task, the animals were excluded when they took longer than 60 s to enter in the dark compartment in the session training.

Data from locomotor activity test and neurotransmitter levels followed a normal distribution (parametric) and are expressed as mean ± standard error of the mean (SEM), while data non-parametric data (inhibitory avoidance test and gene expression) are expressed as interquartile range ± median. For all comparisons, a significance level of $p < 0.05$ was considered. GraphPad Prism 8 (La Jolla, CA, USA) software was used for statistical analysis.

Results

Neurotransmitter Levels Analysis

Since our research group showed behavioral alterations in a previous study of the HD model induced by 3-NPA in adult zebrafish, aiming to understand if the 3-NPA could cause neurochemical alterations, we assessed the neurotransmitter levels. The 3-NPA administration significantly decreased the (a) DA ($p < 0.0004$), (b) GLU ($p < 0.0469$), and (c) 5-HT ($p < 0.0322$) levels in comparison to the control group (Fig. 2).

Effects of 3-NPA Treatment on Dopamine Receptors Gene Expression

To investigate if the 3-NPA could promote changes in gene expression, we also assessed the effects of 3-NPA treatment on dopamine gene expression (Fig. 3). 3-NPA treatment did not significantly alter mRNA transcript levels of *drd1a* ($p = 0.4491$), *drd1b* ($p = 0.5934$), *drd2a* ($p = 0.1552$), and *drd2b* ($p = 0.6505$).

Effect of Dopaminergic Modulator Drugs on Behavioral Parameters in 3-NPA-Treated Adult Zebrafish

Locomotor Activity Test

To test the hypothesis that dopamine modulators drugs can reverse the hypolocomotor effects of 3-NPA treatment, adult zebrafish treated with 3-NPA were submitted to a single injection with the QNP or ETI on day 29. As a result, we obtained the reversion of the hypolocomotion induced by 3-NPA treatment by selective D_2R agonist, QNP (Fig. 4, 3-NPA, $F_{(1,44)} = 6.496$; $p = 0.0144$; QNP, $F_{(1,44)} = 0.2174$; $p = 0.6434$; interaction, $F_{(1,44)} = 40.73$; $p < 0.0001$). In contrast, the selective D_2R antagonist ETI did not reverse the hypolocomotion induced by 3-NPA (Fig. 4, 3-NPA, $F_{(1,44)} = 24.80$; $p < 0.0001$; eticlopride, $F_{(1,44)} = 5.938$; $p = 0.0189$; interaction, $F_{(1,44)} = 25.44$; $p < 0.0001$).

Furthermore, there were no significant locomotor changes in the naïve group (without any manipulation) compared to the control (vehicle) group (Supplementary Fig. 1).

In addition, although we observed that the QNP per se altered the velocity compared to control fish (Supplementary Fig. 2; $p = 0.0004$), no significant difference was found in animals that received QNP or ETI in comparison with fish treated with 3-NPA (Supplementary Fig. 2).

Inhibitory Avoidance Task

Also, we investigated if the dopaminergic drugs could reverse the memory deficit induced by 3-NPA. Interestingly, it was seen that animals treated with QNP (Fig. 5; $p < 0.0002$) had a reversion of memory impairment induced by 3-NPA, as detected by the difference in latencies between the training and test sessions. In contrast, animals treated with ETI did not significantly differ in the latencies between the training and test sessions, indicating that this dopaminergic modulator did not reverse the memory impairment induced by 3-NPA (Fig. 5; $p = 0.8621$).

Fig. 2 Neurotransmitter levels in adult zebrafish whole brain after 3-NPA treatment. Data are expressed as ng/mg of protein, demonstrated as mean ± S.E.M. of six experiments (six whole brain pool) and analyzed by Student's *t*-test. *$p < 0.05$, ***$p < 0.001$
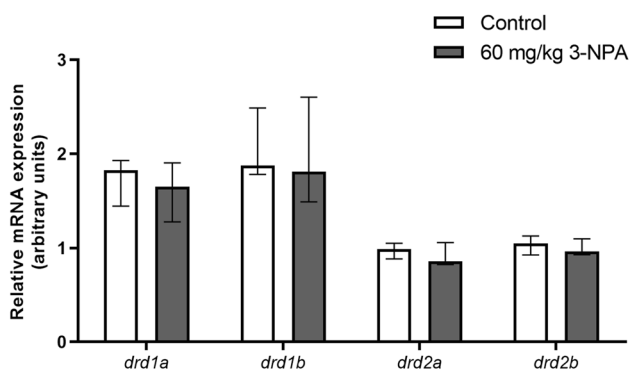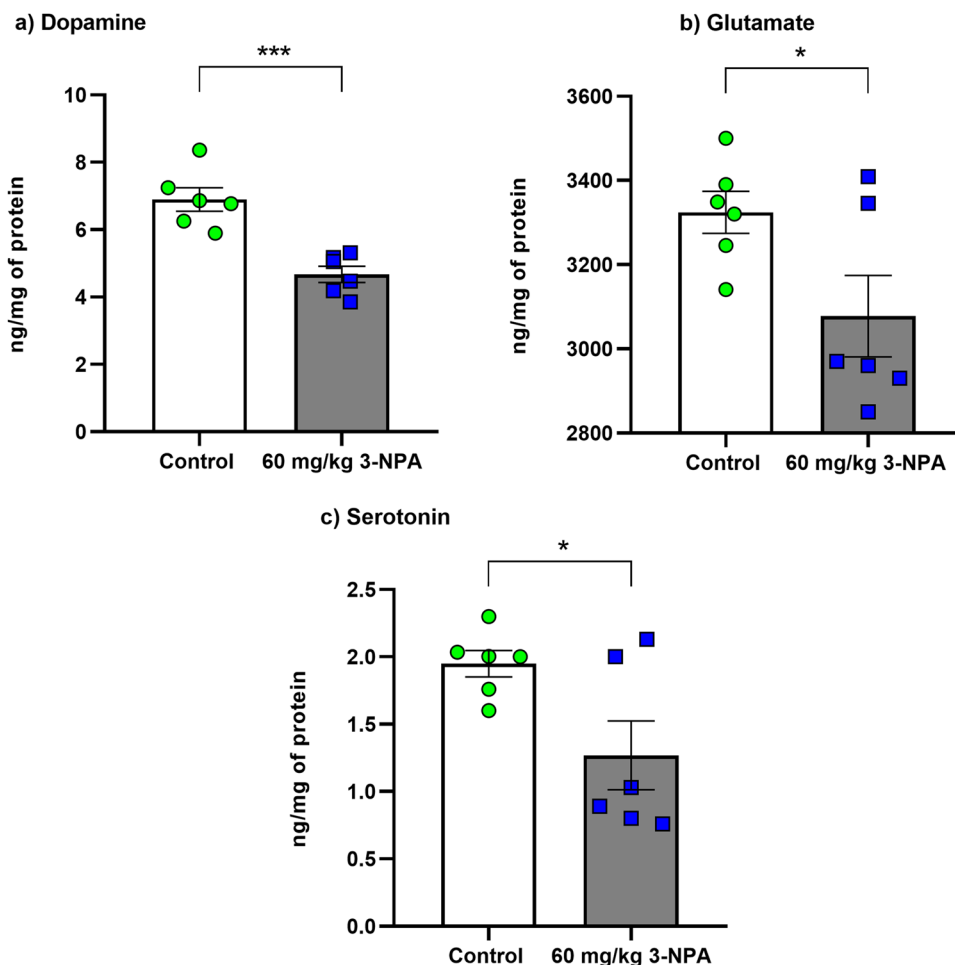
a) Dopamine

b) Glutamate

c) Serotonin

Fig. 3 Analysis of dopamine receptor gene expression in adult zebrafish whole brains treated with 3-NPA or saline. Data are expressed as the interquartile range ± median of six independent experiments (pool of five whole brains) and were evaluated by the Mann–Whitney *U* test

Discussion

Here we showed that 3-NPA decreased the DA, GLU, and 5-HT levels and did not alter the D_1R and D_2R gene expression. Also, we observed that the treatment with the selective D_2R dopamine receptor agonist QNP reversed the bradykinesia-like effects and memory deficits induced by 3-NPA.

Systemic administration of 3-NPA is a gold standard model to induce a spectrum of pathophysiological features of HD because it reproduces both hyperkinetic and hypokinetic and memory clinical symptoms of disease, besides causing changes in neurochemical parameters similar to those found in HD [19, 40]. Several studies in animal models have demonstrated that the 3-NPA chronic administration causes behavioral deficits linked to late-stage HD [18, 40–42]. In this study, 3-NPA chronic treatment in adult zebrafish promoted locomotor dysfunction as evidenced by a decrease in distance traveled in the aquarium, and it caused memory deficit. Following the 3-NPA treatment in adult zebrafish, these behavioral alterations have already been described in a previous study conducted by our research group

Fig. 4 Effects of quinpirole (QNP, 5 mg/kg) treatment, a selective D_2 dopamine agonist, and eticlopride (ETI, 0.1 mg/kg) treatment, a selective D_2 dopamine antagonist on locomotion after 3-NPA treatment. Data are shown as mean ± S.E.M. ($n = 12$ for each group) and were evaluated by two-way ANOVA followed by a Bonferroni post hoc test. ***$p < 0.001$, ****$p < 0.0001$
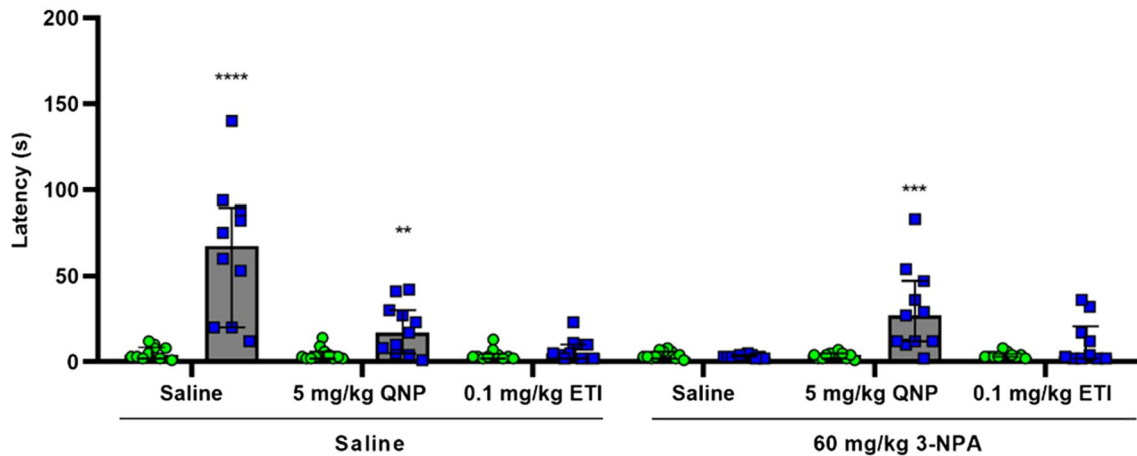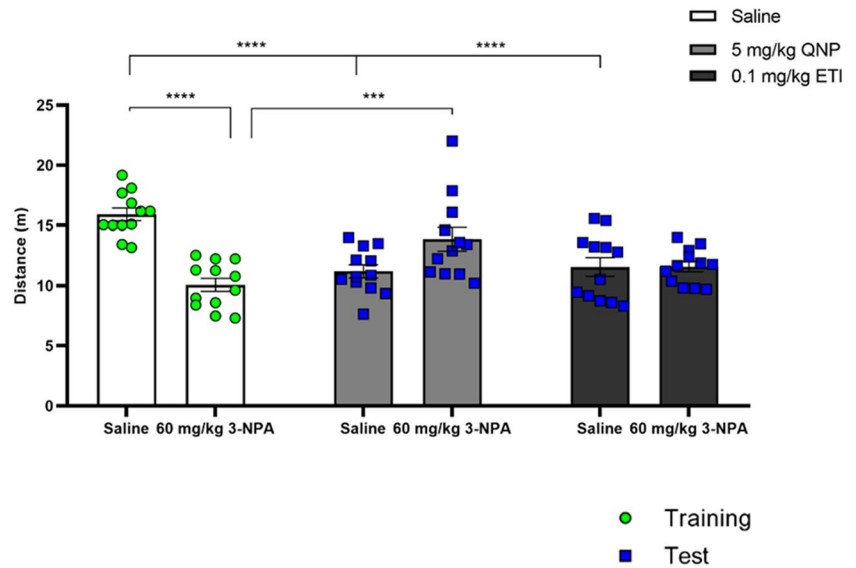
Fig. 5 Effect of selective dopamine receptor agonist quinpirole (QNP, 5 mg/kg) and selective dopamine antagonist eticlopride (ETI, 0.1 mg/kg) treatment on the 3-NPA-induced memory deficit in the inhibitory avoidance on the training and test sessions. Data are shown as the median ± interquartile range ($n = 10$–11 for each group) and were

analyzed individually for each group. **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ show the differences between training and test sessions for each group analyzed by Mann–Whitney U matched pair test. There were no differences between training performances among all groups, as analyzed by the Kruskal–Wallis test

[20]. Thus, the present results are in concordance with earlier studies using 3-NPA in HD animal models. In addition, these behavioral abnormalities may result from neurochemical imbalance, including a depletion in DA levels.

Studies reported that neurotransmission systems imbalance in both HD subjects and HD experimental models. Post-mortem brain HD patients showed an increase in DA levels in the early stage of pathology, while in the late stage of the disease, the DA levels are diminished [9]. In cerebrospinal fluid of HD subjects in the early stage presenting chorea also was found an increase in DA levels [10]. Likewise, a reduction in DA levels was reported in HD subjects in the late stage [43, 44]. Furthermore, in HD patients, the 5-HT levels are increased in the late stage [43]. Similarly, as occurs with the DA levels, studies showed that in GLU neurotransmission happens biphasic changes characterized

by an increase in GLU levels in the early stage and a reduction in the late stage of the disease [45, 46]. Previous studies demonstrated that 3-NPA treatment reproducing the late-stage HD reduces the catecholamine levels, including the DA and 5-HT [40, 41, 47, 48]. An exciting study demonstrated by magnetic resonance imaging that 3-NPA administration in rats induced lesions mainly in striatal projections, which are more responsive to DA [49]. Based on these reports, and to better characterize our previous study validating the HD model induced by 3-NPA [20], in the present study, we evaluated if the 3-NPA could induce neurochemical changes similar to those observed in the late stage of HD. A reduction in DA and 5-HT levels was observed following the 3-NPA treatment in adult zebrafish. We also showed a reduction in GLU levels in adult zebrafish treated with 3-NPA. Contrary to our findings regarding the GLU

levels, studies have observed an increase in GLU levels after the 3-NPA treatment [40, 48]. Our data are in harmony with the literature demonstrating that the 3-NPA treatment has the potential to induce a neurotransmission system imbalance, such as observed in the late stage of HD.

Furthermore, as stated previously, there were no significant locomotor changes in the naïve group (without any manipulation) compared to the control (vehicle) group. This was an interesting result because it demonstrates that repeated i.p. injections by itself did not impair the locomotor activity of animals. Moreover, we did not observe an inflammatory process in the histological analysis in both control and 3-NPA groups (data submitted for publication), reinforcing the idea that manipulation with i.p. repeated injection did not promote significant changes in behavioral parameters. Another point to be discussed is the high death rate (25%) of the animals observed after saline and 3-NPA treatment. It is possible to suggest that the repeated i.p. injections may induce some inflammatory mechanism that could impact more sensitive animals. However, we chose this pathway to 3-NPA treatment because most of the studies in other animal models administered 3-NPA only via i.p. [18, 19, 40–42].

Another neuropathological feature observed in HD is the change in dopamine receptors. To investigate whether 3-NPA could have the potential to cause transcriptional alterations on the dopamine receptors, we analyzed the gene expression by RT-qPCR in 3-NPA or control animals. Although we demonstrated that DA levels were significantly reduced in animals treated with 3-NPA, we did not observe alterations in the gene expression of *drd1a*, *drd2a*, *drd1b*, and *drd2b*. Consistent with our data, a study in rats treated with 3-NPA demonstrated changes neither in D_1R nor in D_2R [49]. Similarly, in an HD genetic model, rat called BACHD also was not observed alterations in D_1R and D_2R in both the ventral and dorsal striatum [50]. Contrary, in R6/1 mice (another genetic animal model of HD), a study observed that the density of D_1R and D_2R decreased in the striatum in R6/1 mice compared to wild-type mice [51, 52]. In the same line, in R6/2 mice was also seen a decrease in the profile of D_2R and D_1R binding [12]. Another study investigated through mRNA profile analysis if *htt* protein length could alter the gene expression in mice expressing a prolonged N-terminal fragment (964 amino acids) called HD46 and HD100 or full-length (YAC72; 3144 amino acids) mutant *htt* protein. The study demonstrated small reductions in D_2R mRNA in HD100 mice. Additionally, HD46 and HD100 mice also showed changes in the D_1R mRNA levels [53]. It is essential to highlight that these divergent results between our study and some studies mentioned above may be because, in genetic models, the *htt* protein length could affect the capability of expanded poly Q domain to act at the transcriptional level altering the

gene expression, while in pharmacological models did not exist this influence. Thus, our results indicate that 3-NPA treatment did not have the potential to alter the genetic transcription.

Currently, there are no cure methods that change the course of HD. Available pharmacological treatments are only to attenuate the neuropsychiatric and motor symptoms improving the life quality of patients. Most of the pharmacological therapies for HD symptoms act on the dopaminergic system [5, 54]. In addition, the most often used pharmacological options available are for the treatment of chorea, focusing on the early stage of the disease [5, 55]. As first choice drugs are the selective inhibitors of vesicular transporter monoamine (tetrabenazine and deutetrabenazine) followed by a dopaminergic stabilizer (pridopidine). The second-line choice is the dopamine antagonists such as haloperidol, olanzapine, and sulpiride [5, 56] because they can decrease hyperdopaminergic neurotransmission and DA levels, consequently suppressing the chorea by controlling the DA reuptake and blocking postsynaptic dopamine D_2R [57]. In agreement with this, studies showed that the administration of levodopa exacerbated the chorea symptoms [58, 59]. Although there are pharmacological options for treating early symptoms, the pharmacological agents for treating bradykinesia are still scarce, and there is evidence limited to the use of dopamine agonists [60, 61]. In the present study, we observed that QNP (a selective D_2R agonist) treatment reversed the hypolocomotion and memory deficits induced by 3-NPA treatment, while the treatment with ETI (a selective D_2R antagonist) did not reverse the behavioral alterations induced by 3-NPA treatment in adult zebrafish. In agreement with our results, Lukács et al. [34] showed that rats treated with 3-NPA had a decrease in locomotor activity which was reversed by the treatment with the QNP, whereas rats treated with sulpiride (a selective D_2R antagonist) after 3-NPA treatment did not have an increase in their locomotor activity [34]. QNP also reduced the striatal lesion induced by malonate in rats [62]. Furthermore, in fully symptomatic R6/2 mice, the treatment with levodopa in the short-term improved the behavioral alterations in animals HD compared with controls [63]. Studies analyzed the synaptic plasticity in the perirhinal cortex responsible for long-term depression and potentiation in recognition memory in R6/2 mice. The authors observed a delay in both long- and short-term depression plasticity, which was reversed by D_2R dopamine agonist QNP treatment [64]. Therefore, the findings may suggest that the use of DA agonists in the late stage of HD may be beneficial. This therapeutical potential of DA agonists may be because it could be occurring heterodimerization between D_2R and adenosine A_{2A} receptors ($A_{2A}R$); that is, the D_2R activation may be blocking the $A_{2A}R$, and thus modulating

the adenosine levels as well as other neurotransmitter systems. This suggestion is supported by a recent study where the modulation of the adenosinergic system with the selective $A_{2A}R$ agonist CGS 21680 worsened the hypolocomotion and did not reverse the memory decline, whereas the selective $A_{2A}R$ antagonist ZM 241385 reversed the hypolocomotion and the memory decline in adult zebrafish treated with 3-NPA [65].

Another interesting result to be discussed is the effect of QNP and ETI per se on behavioral responses. We observed that QNP and ETI per se decreased locomotor activity. Also, the QNP per se did not affect memory, while ETI per se caused memory impairment. Similarly, evidence demonstrated that the ETI and QNP treatment per se significantly reduced the basal distance traveled [66–68]. Also, rats of both sexes submitted to the D_2R antagonist ETI treatment before cocaine treatment had a decrease in the hyperlocomotion induced by cocaine, while QNP by itself reduced the locomotor activity in both male and female animals, with a decrease more pronounced in male than female rats [69]. Additionally, ETI treatment had no significant effect on the performance in the passive avoidance test and was unable to attenuate the methamphetamine-induced amnesia [67]. Another study investigated the D_2R modulation on the pre-frontal cortex in monkeys observing that the blockade with ETI impaired associative learning and cognitive flexibility, besides decreasing the neural information about the associations [70]. Compatible with our results, zebrafish treated with QNP and ETI in three conditions (before training, after training, and before probe) submitted to a cognitive performance task performed by the plus-maze associative learning paradigm demonstrated an enhancement in learning performance before the training when administered QNP. On the contrary, the animals that received ETI before and after the training demonstrated an impairment in associative learning performance [71]. Furthermore, studies showed that the QNP can enhance memory consolidation and retention, besides reducing the freezing in inhibitory avoidance task [72, 73]. It is important to note that the QNP has a biphasic dose-dependent effect that may result in pre- or postsynaptic D_2R/D_3R activation [74]. Thus, in a neurodegenerative condition, the QNP could act on postsynaptic D_2R, besides blocking the $A_{2A}R$, consequently reducing the cyclic adenosine monophosphate and the protein kinase A, resulting in a neuroprotective effect [75].

Conclusion

In summary, the findings indicate that the 3-NPA effects are directly associated with the dopaminergic system since it was identified deficiency in DA levels, which may be correlated with locomotor and memory deficits induced by 3-NPA in adult zebrafish. Furthermore, the pharmacological activation of D_2R can reverse the locomotor dysfunction and memory impairment induced by 3-NPA in adult zebrafish. Overall, the data corroborate the evidence that the dopaminergic system is altered in the late stage of HD, and the modulation of the dopaminergic system with DA agonists in this stage could be a pharmacological strategy to improve the bradykinesia. Therefore, the DA agonist modulator may be an interesting approach in the late stage of HD. Further research should be conducted to clarify the benefits of DA agonist modulators in HD patients.

Declarations

Ethics Approval All protocols were approved by the Institutional Animal Care Committee from Pontifícia Universidade Católica do Rio Grande do Sul (CEUA-PUCRS, permit number 9406/2019) and comply with the guideline 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).

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

References

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes (1993) The huntington's disease collaborative research group. Cell 72:971–983

2. Capiluppi E, Romano L, Rebora P, Nanetti L, Castaldo A, Gellera C, Mariotti C, Macerollo A et al (2020) Late-onset Huntington's disease with 40–42 CAG expansion. Neurol Sci 41:869–876

3. Cepeda C, Levine MS (2022) Synaptic dysfunction in Huntington's disease: lessons from genetic animal models. Neuroscientist 28(1):20–40

4. Coppen EM, Jacobs M, van den Berg-Huysmans AA, van der Grond J, Roos RAC (2018) Grey matter volume loss is associated with specific clinical motor signs in Huntington's disease. Parkinsonism Relat Disord 46:56–61

5. Coppen EM, Roos RAC (2017) Current pharmacological approaches to reduce chorea in Huntington's disease. Drugs 77(1):29–46

6. Stahl CM, Feigin A (2020) Medical, surgical, and genetic treatment of Huntington disease. Neurol Clin 38:367–378

7. Barry J, Bui MTN, Levine MS, Cepeda C (2022) Synaptic pathology in Huntington's disease: beyond the corticostriatal pathway. Neurobiol Dis 162:105574

8. Klein MO, Battagello DS, Cardoso AR, Hauser DN, Bittencourt JC, Correa RG (2019) Dopamine: functions, signaling, and association with neurological disease. Cell Mol Neurobiol 39(1):31–59

9. Bird ED, Iversen LL (1974) Huntington's chorea. Post-mortem measurement of glutamic acid decarboxylase, choline acetyltransferase and dopamine in basal ganglia. Brain 97(3):457–472

10. Garret MC, Soares-da-Silva P (1992) Increased cerebrospinal fluid dopamine and 3,4-dihydroxyphenylacetic acid levels in Huntington's disease: evidence for an overactive dopaminergic brain transmission. J Neurochem 58(1):101–110

11. Dallérac GM, Levasseur G, Vatsavayai SC, Milnerwood AJ, Cummings DM, Kraev I, Huetz C, Evans KA et al (2015) Dysfunctional dopaminergic neurons in mouse models of Huntington's disease: a role for SK3 channels. Neurodegener Dis 15(2):93–108

12. Cha JH, Kosinski CM, Alsdorf SA, Mangiarini L, Davies SW, Penney JB, Bates GP, Young AB (1998) Altered brain neurotransmitter receptors in transgenic mice expressing a portion of an abnormal human Huntington disease gene. Proc Natl Acad Sci U S A 95(11):6480–6485

13 Graybiel AM (2000) The basal ganglia. Curr Bio l10:R509–R511

14. Brouillet E, Condé F, Beal MF, Hantraye P (1999) Replicating Huntington's disease phenotype in experimental animals. Prog Neurobiol 59:427–468

15. Beal MF (1992) Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? Ann Neurol 31:119–130

16. Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AHV (1996) Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol 39:385–389

17. Brouillet E, Guyot MC, Mittoux V, Altairac S, Condé F, Palfi S, Hantraye P (1998) Partial inhibition of brain succinate dehydrogenase by 3-nitropropionic acid is sufficient to initiate striatal degeneration in rat. J Neurochem 70:794–805

18. Bortolatto CF, Reis AS, Pinz MP, Voss GT, Oliveira RL, Vogt AG, Roman S, Jesse CR et al (2017) Selective A2A receptor antagonist SCH 58261 modulates striatal oxidative stress and alleviates toxicity induced by 3-nitropropionic acid in male Wistar rats. Metab Brain Dis 32:1919–1927

19. Borlongan CV, Koutouzis TK, Freeman TB, Hauser RA, Cahill DW, Sanberg PR (1997) Hyperactivity and hypoactivity in a rat model of Huntington's disease: the systemic 3-nitropropionic acid model. Brain Res Brain Res Protoc 1:253–257

20. Wiprich MT, Zanandrea R, Altenhofen S, Bonan CD (2020) Influence of 3-nitropropionic acid on physiological and behavioral responses in zebrafish larvae and adults. Comp Biochem Physiol C Toxicol Pharmacol 234:108772

21. Kiper K, Freeman JL (2021) Use of zebrafish genetic models to study etiology of the amyloid-beta and neurofibrillary tangle pathways in Alzheimer's disease. Curr Neuropharmacol 20(3):524–39

22. Razali K, Othman N, Mohd Nasir MH, Doolaanea AA, Kumar J, Ibrahim WN, Mohamed Ibrahim N, Mohamed W (2021) The promise of the zebrafish model for Parkinson's disease: today's science and tomorrow's treatment. Front Genet 12:655550

23. Chakraborty HJ, Rout AK, Behera BK, Parhi J, Parida PK, Das BK (2018) Insights into the aquaporin 4 of zebrafish (Danio rerio) through evolutionary analysis, molecular modeling and structural dynamics. Gene Rep 11:101–109

24. Rink E, Wullimann MF (2001) The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). Brain Res 889(1–2):316–330

25. Lv DJ, Li LX, Chen J, Wei SZ, Wang F, Hu H, Xie AM, Liu CF (2019) Sleep deprivation caused a memory defects and emotional changes in a rotenone-based zebrafish model of Parkinson's disease. Behav Brain Res 372:112031

26. Thörnqvist PO, McCarrick S, Ericsson M, Roman E, Winberg S (2019) Bold zebrafish (Danio rerio) express higher levels of delta opioid and dopamine D2 receptors in the brain compared to shy fish. Behav Brain Res 359:927–934

27. Paiva IM, Sartori BM, Castro TFD, Lunkes LC, Virote BCR, Murgas LDS, de Souza RP, Brunialti-Godard AL (2020) Behavioral plasticity and gene regulation in the brain during an intermittent ethanol exposure in adult zebrafish population. Pharmacol Biochem Behav 192:172909

28. Westerfield M (2000) The zebrafish book. A guide for the laboratory use of zebrafish (Danio rerio), 4th edn. University of Oregon Press, Eugene

29. Petersen BD, Pereira TCB, Altenhofen S, Nabinger DD, Ferreira PMA, Bogo MR, Bonan CD (2021) Antibiotic drugs alter zebrafish behavior. Comp Biochem Physiol C Toxicol Pharmacol 242:108936

30. Mocelin R, Marcon M, Araujo ASR, Herrmann AP, Piato A (2019) Withdrawal effects following repeated ethanol exposure are prevented by N-acetylcysteine in zebrafish. Prog Neuropsychopharmacol Biol Psychiatry 93:161–170

31. Giacomini ACVV, Piassetta AS, Genario R, Bonan CD, Piato A, Barcellos LJG, de Abreu MS (2020) Tryptophan alleviates neuroendocrine and behavioral responses to stress in zebrafish. Behav Brain Res 378:112264

32. Altenhofen S, Nabinger DD, Pereira TCB, Leite CE, Bogo MR, Bonan CD (2018) Manganese(II) chloride alters nucleotide and nucleoside catabolism in zebrafish (Danio rerio) adult brain. Mol Neurobiol 55(5):3866–3874

33. Zanandrea R, Wiprich MT, Altenhofen S, Rubensam G, Dos Santos TM, Wyse ATS, Bonan CD (2020) Withdrawal effects following methionine exposure in adult zebrafish. Mol Neurobiol 57:3485–3497

34. Lukács A, Szabó A, Papp A, Vezér T (2009) Altered open field behavior in rats induced by acute administration of 3-nitropropionic acid: possible glutamatergic and dopaminergic involvement. Acta Biol Hung 60(4):359–367

35. Taylor JL, Bishop C, Walker PD (2005) Dopamine D1 and D2 receptor contributions to L-DOPA-induced dyskinesia in the dopamine-depleted rat. Pharmacol Biochem Behav 81(4):887–893

36. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

37. Pfaff MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45

38. Tang R, Dodd A, Lai D, McNabb WC, Love DR (2007) Validation of zebrafish (Danio rerio) reference genes for quantitative real-time RT-PCR normalization. Acta Biochim Biophys Sin (Shanghai) 39:384–390

39. Blank M, Guerim LD, Cordeiro RF, Vianna MRM (2009) A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. Neurobiol Learn Mem 92:529–534

40. Kaur N, Jamwal S, Deshmukh R, Gauttam V, Kumar P (2015) Beneficial effect of rice bran extract against 3-nitropropionic acid induced experimental Huntington's disease in rat. Toxicol Rep 2:1222–1232

41. Kumar P, Kumar A (2009) Possible role of sertraline against 3-nitropropionic acid induced behavioral, oxidative stress and mitochondrial dysfunctions in rat brain. Prog Neuropsychopharmacol Biol Psychiatry 33(1):100–108

42. Salman M, Sharma P, Alam MI, Tabassum H, Parvez S (2021) Naringenin mitigates behavioral alterations and provides neuroprotection against 3-nitropropinoic acid-induced Huntington's disease like symptoms in rats. Nutr Neurosci 15:1–11

43. Bernheimer A, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F (1973) Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J Neurol Sci 20(4):415–455

44. Kish SJ, Shannak K, Hornykiewicz O (1987) Elevated serotonin and reduced dopamine in subregionally divided Huntington's disease striatum. Ann Neurol 22(3):386–389

45. Klapstein GJ, Fisher RS, Zanjani H, Cepeda C, Jokel ES, Chesselet MF, Levine MS (2001) Electrophysiological and morphological changes in striatal spiny neurons in R6/2 Huntington's disease transgenic mice. J Neurophysiol 86(6):2667–2677

46. Andre VM, Cepeda C, Fisher YE, Huynh M, Bardakjian N, Singh S, Yang XW, Levine MS (2011) Differential electrophysiological changes in striatal output neurons in Huntington's disease. J Neurosci 31(4):1170–1182

47. Denny Joseph KM, Muralidhara, (2014) Neuroprotective efficacy of a combination of fish oil and ferulic acid against 3-nitropropionic acid-induced oxidative stress and neurotoxicity in rats: behavioral and biochemical evidence. Appl Physiol Nutr Metab 39(4):487–496

48. Jamwal S, Kumar P (2016) Spermidine ameliorates 3-nitropropionic acid (3-NP)-induced striatal toxicity: possible role of oxidative stress, neuroinflammation, and neurotransmitters. Physiol Behav 155:180–187

49. Binienda ZK, Ali SF, Virmani A, Amato A, Salem N, Przybyla BD (2006) Co-regulation of dopamine D1 receptor and uncoupling protein-2 expression in 3-nitroproionic acid-induced neurotoxicity: neuroprotective role of L-carnitine. Neurosci Lett 410(1):62–65

50. Manfré G, Novati A, Faccini I, Rossetti AC, Bosch K, Molteni R, Riva MA, Van der Harst JE et al (2018) BACHD rats expressing full-length mutant huntingtin exhibit differences in social behavior compared to wild-type littermates. PLoS One 13(2):e0192289

51. Dowie MJ, Bradshaw HB, Nicholson LFB, Faull RLM, Hannan AJ, Glass M (2009) Altered CB1 receptor and endocannabinoid levels precede motor symptom onset in a transgenic model of Huntington's disease. Neuroscience 163(1):456–465

52. Harris KL, Mason SL, Vallin B, Barker RA (2022) Reduced expression of dopamine D2 receptors on astrocytes in R6/1 mice and HD post-mortem tissue. Neurosci Lett 767:136289

53. Chan EYW, Luthi-Carter R, Strand A, Solano MS, Hanson SA, DeJohn MM, Kooperberg C, Chase KO et al (2002) Increased huntingtin protein length reduces the number of polyglutamine-induced gene expression changes in mouse models of Huntington's disease. Hum Mol Genet 11(17):1939–1951

54. Kumar A, Kumar V, Singh K, Kumar S, Kim YS, Lee YN, Kim JJ (2020) Therapeutic advances for Huntington's disease. Brain Sci 10(1):43

55. Jablonska M, Grzelakowska K, Wisniewski B, Mazur E, Leis K, Galazka P (2020) Pridopidine in the treatment of Huntington's disease. Rev Neurosci 31(4):441–451

56. Kim A, Lalonde K, Truesdell A, Welter PG, Brocardo PS, Rosenstock TR, Gil-Mohapel J (2021) New avenues for the treatment of Huntington's disease. Int J Mol Sci 22(16):8363

57. Venuto CS, McGarry A, Ma Q, Kieburtz K (2012) Pharmacological approaches to the treatment of Huntington's disease. Mov Disord 27(1):31–41

58. Gerstenbrand F, Pateisky K, Prosenz P (1963) Experiences with L-dopa in the therapy of parkinsonism. Psychiatr Neurol (Basel) 146:246–261

59 Klawans HL Jr (1970) A pharmacologic analysis of Huntington's chorea. Eur Neurol 4(3):148–163

60 Racette BA, Perlmutter JS (1998) Levodopa responsive parkinsonism in an adult with Huntington's disease. J Neurol Neurosurg Psychiatry 65(4):577–579

61. Bonelli RM, Wenning GK (2006) Pharmacological management of Huntington's disease: an evidence-based review. Curr Pharm Des 12(21):2701–2720

62. Armentero MT, Fancellu R, Nappi G, Blandini F (2002) Dopamine receptor agonists mediate neuroprotection in malonate-induced striatal lesion in the rat. Exp Neurol 178(2):301–305

63. Hickey MA, Reynolds GP, Morton AJ (2002) The role of dopamine in motor symptoms in the R6/2 transgenic mice model of Huntington's disease. J Neurochem 81:46–59

64. Cummings DM, Milnerwood AJ, Dallérac GM, Waights V, Brown JY, Vatsavayai SC, Hirst MC, Murphy KPSJ (2006) Aberrant cortical synaptic plasticity and dopaminergic dysfunction in a mouse model of Huntington's disease. Hum Mol Genet 15(19):2856–2868

65. Wiprich MT, Altenhofen S, Gusso D, Vasques RR, Zanandrea R, Kist LW, Bogo MR, Bonan CD (2022) Modulation of adenosine signaling reverses 3-nitropropionic acid-induced bradykinesia and memory impairment in adult zebrafish. Prog Neuropsychopharmacol Biol Psychiatry 119:110602

66. Stiles L, Zheng Y, Darlington CL, Smith PF (2012) The D2 dopamine receptor and locomotor hyperactivity following bilateral vestibular deafferentation in the rat. Behav Brain Res 227:150–158

67. Wong YK, Chou MK, Shen YC, Wang YH, Yen JC, Chen CF, Lin SK, Liao JF (2014) Preventive effect of baicalein on methamphetamine-induced amnesia in the passive avoidance test in mice. Pharmacology 93:278–285

68. Pértile RAN, Corvino ME, Marchette RCN, Pavesi E, Cavalli J, Ramos A, Izídio GS (2017) The quinpirole hypolocomotive effects are strain and route administration dependent in SHR and SLA16 isogenic rats. Behav Genet 47:552–563

69. Schindler CW, Carmona GN (2002) Effects of dopamine agonists and antagonists on locomotor activity in male and female rats. Pharmacol Biochem Behav 72:857–863

70. Puig MC, Miller EK (2015) Neural substrates of dopamine D2 receptor modulated executive functions in the monkey prefrontal cortex. Cereb Cortex 25:2980–2987

71. Naderi M, Jamwal A, Chivers DP, Niyogi S (2016) Modulatory effects of dopamine receptors on associative learning performance in zebrafish (Danio rerio). Behav Brain Res 303:109–119

72. de Oliveira AR, Reimer AE, Brandão ML (2006) Dopamine D2 receptor mechanisms in the expression of conditioned fear. Pharmacol Biochem Behav 84:102–111

73. Lénárd L, Ollmann T, László K, Kovács A, Gálosi R, Kállai V, Attila T, Kertes E et al (2017) Role of D2 dopamine receptors of the ventral pallidum in inhibitory avoidance learning. Behav Brain Res 321:99–105

74. Li SM, Collins GT, Paul NM, Grundt P, Newman AH, Xu M, Grandy DK, Woods JH et al (2010) Yawning and locomotor behavior induced by dopamine receptor agonists in mice and rats. Behav Pharmacol 21:171–181

75. Shang P, Baker M, Banks S, Hong SI, Choi DS (2021) Emerging nondopaminergic medications for Parkinson's disease: focusing on A_{2A} receptor antagonists and GLP1 receptor agonists. J Mov Disord 14:193–203