Comparative Biochemistry and Physiology, Part C 234 (2020) 108772

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

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

Influence of 3-nitropropionic acid on physiological and behavioral responses in zebrafish larvae and adults

Melissa Talita Wiprich[a,b], Rodrigo Zanandrea[a,b], Stefani Altenhofen[a,b,c], Carla Denise Bonan[a,b,c,*]

[a] 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
[b] Laboratório de Neuroquímica e Psicofarmacologia, Escola de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Instituto Nacional de Ciência e Tecnologia em Doenças Cerebrais, Excitotoxicidade e Neuroproteção, Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:
Locomotion
3-Nitropropionic acid
Huntington's disease
Memory
Zebrafish

ABSTRACT

Long-term treatment with 3-nitropropionic acid (3-NPA), a toxin derived from plants and fungi, may reproduce symptoms and biochemical characteristics of Huntington's disease (HD). Our study evaluated the effects of 3-NPA on the physiological and behavioral responses in zebrafish larvae and adults. Larvae exposed to 0.1, 0.2, or 0.5 mM 3-NPA exhibited an increase in heart rate at 2- and 5-days post-fertilization (dpf). There was a decrease in the ocular distance at 5 dpf with 0.05 mM 3-NPA treatment. However, 3-NPA did not alter larval locomotor parameters. Adult zebrafish received 3-NPA intraperitoneal injections (a total of seven injections at doses 10, 20, or 60 mg/kg every 96 h) and showed a decrease in body weight , locomotion and aggressive behavior. No changes were observed in anxiety-like behavior and social interaction between 3-NPA-exposed animals and control groups. However, 3-NPA-treated animals (at 60 mg/kg) demonstrated impaired long-term aversive memory. Overall, 3-NPA exposure induced morphological and heart rate alterations in zebrafish larvae. Additionally, our study showed behavioral changes in zebrafish that were submitted to long-term 3-NPA treatment, which could be related to HD symptoms.

1. Introduction

Huntington's disease (HD) is a fatal, autosomal dominant neurodegenerative disorder caused by an expansion of a CAG triplet repeat in exon 1 of the huntingtin (*htt*) gene. This change leads to an expanded polyglutamine (polyQ) region in the encoded protein *htt* (Bailus et al., 2017; Rai et al., 2019). It is estimated that the mean HD prevalence is 5 in 100,000 people (Baig et al., 2016; Illarioshkin et al., 2018). HD is characterized by progressive motor dysfunction (chorea, bradykinesia, and dystonia), psychiatric disturbance, and cognitive decline (Blum et al., 2018; Mason et al., 2018). These dysfunctions can be attributed to multiple brain regions that exhibit neurodegeneration, including the cerebral cortex, thalamus, subthalamic nucleus, globus pallidus, substantia nigra, and hypothalamus. However, the hallmark of the disease is the pronounced neuronal loss in the striatum (caudate nucleus and putamen; Rubinsztein, 2002; Ramaswamy et al., 2007; Coppen and Roos, 2017). Furthermore, HD patients may develop metabolic symptoms such as weight loss, cardiac dysfunction, and insulin resistance (Blum et al., 2018; Croce and Yamamoto, 2018; Dufour and McBride, 2019). The remarkable anatomical and physiological similarities between humans and animals allow researchers to investigate a wide range of mechanisms and develop novel therapies using animal models before applying their discoveries to humans. The HD animal models include approaches that span from pharmacological-induced models to refined strategies to develop genetically-modified experimental animals (Kaur et al., 2017). Genetic models have been developed in several species (Chan et al., 2015; Schuldenzucker et al., 2017; Morton, 2018; Stricker-Shaver et al., 2018), and studies in huntingtin-deficient zebrafish show impaired neuronal development, morphological alterations, and hematological defects during the embryo-larval stage (Lumsden et al., 2007; Diekmann et al., 2009). However, there is no behavioral analysis in the larval and adult stages (Lumsden et al., 2007; Diekmann et al., 2009).

The great advantage of the pharmacological HD models is their ease of use, control, and acquisition of drugs that can induce HD-like symptoms. Among them, the most used is 3-nitropropionic acid (3-NPA), a natural toxin produced by fungi and plants. Several studies demonstrated that there is a decrease in mitochondria, calcium, ATP and mitochondrial complex II, III and IV activity in the striatum of HD patients (Gu et al., 1996; Johri et al., 2013; Carmo et al., 2018).

* 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 – Prédio 12, bloco D, sala 301, Porto Alegre, RS, Brazil.
E-mail address: cbonan@pucrs.br (C.D. Bonan).

https://doi.org/10.1016/j.cbpc.2020.108772
Received 17 January 2020; Received in revised form 9 April 2020; Accepted 18 April 2020
Available online 27 April 2020

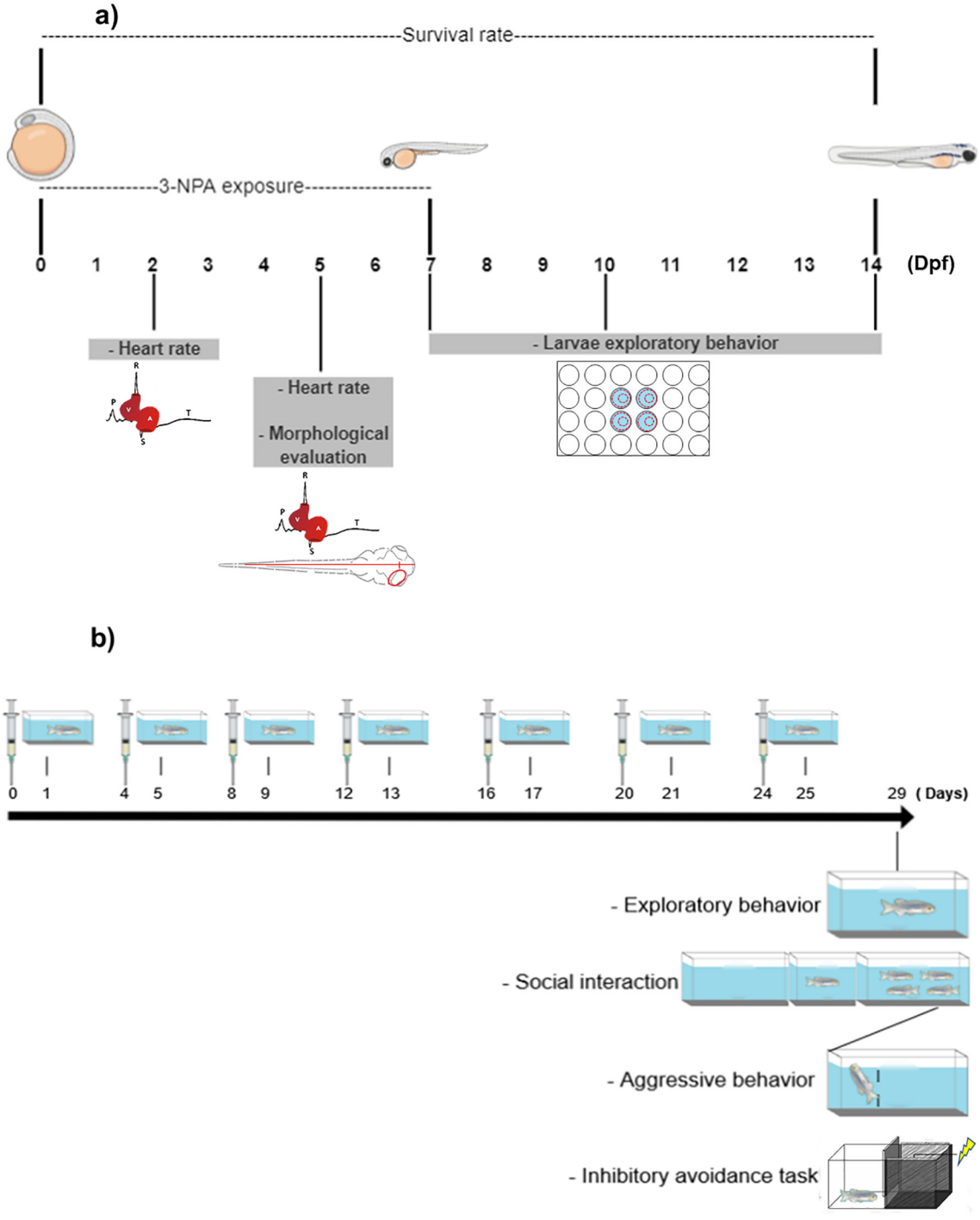

Fig. 1. Timeline of the experimental procedure. (a) Zebrafish larvae were exposed to water (control group) or 0.01, 0.05, 0.1, 0.2, 0.5, 1.0 or 2.0 mM 3-Nitropropionic acid (3-NPA). The exposure occurred from 0 dpf (day post-fertilization) to 7 days post-fertilization. The analysis lasted 14 days post-fertilization and included survival rate, heart rate, morphological evaluation, and exploratory behavior. (b) Zebrafish adults received seven intraperitoneal injections of 10, 20, or 60 mg/kg 3-Nitropropionic acid or saline (vehicle-control group). The injections occurred over 28 days, and the analysis included a novel tank test, social interaction, aggression, and inhibitory avoidance task.

Interestingly, 3-NPA promotes irreversible inhibition of the enzyme succinate dehydrogenase (SDH) of mitochondrial complex II of the respiratory chain. Furthermore, 3-NPA can cross the blood-brain barrier (BBB) and therefore damage the central nervous system (CNS) even after systemic administration (Brouillet et al., 1999; Túnez and Santamaría, 2009; Túnez et al., 2010; Kaur et al., 2017).

Accidental ingestion of 3-NPA in humans causes cell death in the bilateral putamen and caudate nucleus and motor impairment characterized as dystonia, involuntary spasms, and facial grimaces (Ludolph et al., 1991; Ming, 1995). In rodents, acute and chronic treatment with 3-NPA mimics the motor symptoms and cellular alterations observed in HD. These dysfunctions include ataxia, choreiform movements, insanity, bradykinesia, muscle weakness, rigidity, weight loss, SDH inhibition, oxidative stress, and mitochondrial dysfunction, among others (Kim et al., 2000; Fernagut et al., 2002; Bortolatto et al., 2017; Kaur et al., 2017).

In this context, the zebrafish is an excellent animal model for studying and developing neurodegenerative disease models due to the myriad advantages over other animal models. These benefits include a relatively low cost, fast development and large progenies, ease of genetic manipulation, and physiology that is highly similar to humans (Goldsmith, 2004; Rico et al., 2011; Howe et al., 2013; Fontana et al., 2018; Vaz et al., 2018). The zebrafish CNS is also well characterized; brain development occurs within 3 days post fertilization (dpf), and the BBB develops from 3 to 10 dpf (Fleming et al., 2013; d'Amora and Giordani, 2018; Vaz et al., 2018; Quiñonez-Silvero et al., 2019). Additionally, the behavioral repertoire of this species associated with its high drug sensitivity permits its use as a platform for mechanistic and therapeutic research (Stewart et al., 2014; Da Silva et al., 2015; Baxendale et al., 2017).

Given these advantages, the present study evaluated the effects of 3-NPA on the behavioral responses of zebrafish (*Danio rerio*) in larval and adult stages. Thus, the effects of this compound on morphology, exploratory behavior, aggression, social interaction, and cognition were investigated.

2. Materials and methods

2.1. Animals

Embryos and larvae (0–14 dpf) and adult (6–14 months, 0.2–0.4 g) wild-type *Danio rerio,* from the AB background, were used. Animals were obtained from our breeding colony and maintained in recirculating systems (Zebtec, Tecniplast, Italy) with equilibrated filtered water to reach the species standard temperature (28 ± 2 °C), pH (7.0 to 7.5), conductivity (300–700 μS), and ammonia (< 0.02 mg/L), nitrite (< 1 mg/L), nitrate (< 50 mg/L), and chloride (0 mg/L) levels. Animals were subjected to a light/dark cycle of 14/10 h, respectively. Animals received paramecium between 6 and 14 dpf, and after 14 dpf, they received commercial flakes (TetraMin Tropical Flake Fish®) three times a day that were supplemented with brine shrimp (Westerfield, 2000).

For breeding, females and males (1:2) were placed in breeding tanks (Tecniplast, Italy) overnight. They were separated by a transparent barrier that was removed after the lights went on the following morning. For the larval experiments, embryos were collected, sanitized, and subjected to the treatment. For the experiments with adult animals, the embryos were collected and maintained for up to 7 dpf at a density of 1 larva per 7 mL in Petri dishes in a biochemical oxygen demand (BOD) incubator. They were immediately transferred to a tank in the recirculation system with a density of 1 larva per 60 mL. When the animals reached 30 dpf, they were maintained at a density of 1 animal per 200 mL until adulthood. All protocols were approved by the Animal Care Committee of the Pontifical Catholic University of Rio Grande do Sul (8024- CEUA- PUCRS). This study was registered in the Sistema Nacional de Gestão do Patrimônio Genético e Conhecimento Tradicional Associado - SISGEN (Protocol No. A3B073D).

2.2. Treatments

2.2.1. Larval treatment

Embryos were placed in Petri dishes (60 × 16 mm) and subjected to 3-NPA ($O_2N(CH_2)CO_2H$; Sigma-Aldrich, St. Louis, MO, USA) treatment at the concentrations of 0.01, 0.05, 0.1, 0.2, 0.5, 1.0, or 2.0 mM (Colle et al., 2012) diluted in water for 7 dpf (2 h post fertilization [hpf] to 7 dpf; Fig. 1a) without medium changes. These concentrations were chosen based on a previous study (Colle et al., 2012), which tested 3-NPA in cortical and striatal neurons. The control group was exposed to water and maintained under the same conditions as the treatment group. Importantly, animals exposed to concentrations of 1.0 and 2.0 mM 3-NPA died over the 7 days. For this reason, these concentrations were excluded in the subsequent experiments.

The pH of the solution was adjusted between 7.2 and 7.6 with sodium hydroxide (NaOH) and was verified daily. 3-NPA is very soluble in water. During the exposure period, the medium was not changed to minimize the animal's stress. There was no appearance of crystals or odor changes, and the pH remained between 7.2 and 7.6 at all tested 3-NPA concentrations. From 7 to 14 dpf, the animals only remained in the water and the medium was changed daily. Animals were monitored daily for survival, determined by the presence of a heartbeat visualized using an inverted stereomicroscope (Nikon, Melville, EUA).

2.2.2. Adult animal treatment

Adult animals of both sexes, aged between 6 and 10 months, received seven intraperitoneal (i.p.) injections of 3-NPA or saline (vehicle, for the control group) every 96 h (Fig. 1b). Prior to injection, the animals were anesthetized by immersion in 250 mg/L tricaine solution until they showed a lack of motor coordination and a reduced respiration rate. The animals received an i.p. injection of 10, 20 or 60 mg/kg body weight 3-NPA or saline (vehicle-control group) in a 10-μL volume. We selected these doses from other studies testing the effects of 3-NPA in rodents, which demonstrated behavioral changes in acute and chronic exposure to 3-NPA (Borlongan et al., 1995; Fernagut et al., 2002).

After the injection, the animals were placed in a tank with a highly aerated recirculation system to facilitate their recovery from anesthesia. After recovery, the animals were returned to their home tank. I.p. injections were conducted using a 3/10-mL U-100 BD Ultra- Fine™ Short Insulin Syringe with an 8 mm (5/16 in) × 31 G short needle (Becton Dickinson and Company, New Jersey, USA).

2.3. Larval analysis

2.3.1. Heart rate

Heart rate measurement was performed at 2 and 5 dpf using a stereomicroscope. Treated larvae and the controls were placed in 96-well plates (1 larva per plate), and their heart rates were monitored for 60 s (n = 30). For all groups, water or treatment temperature was kept stable at 28 °C using a thermoplate coupled to the stereomicroscope (Macchi et al., 2018; Nabinger et al., 2018).

2.3.2. Morphological evaluation

Morphological evaluation was performed by monitoring morphological defects using a stereomicroscope (3× magnification) at 5 dpf (n = 30). Body length (μm), ocular distance (μm), and surface area of the eyes (μm^2) were evaluated using NIS-Elements D software for Windows 3.2 (Nikon Instruments Inc., Melville, USA). The animals were placed in the dorsal position in 96-well plates (1 larva per plate). Body length was estimated according to Altenhofen et al. (2017); the distance from the larval mouth to the pigmented tip of the tail was measured. The ocular distance was evaluated by the distance between the inner edge of the two eyes (similar to the inner intercanthal distance in humans), and the size of the eyes was determined by measuring the surface area of the eyes (Lutte et al., 2015; Altenhofen et al., 2017).

2.3.3. Exploratory behavior

Larval exploratory behavior was evaluated at 7, 10, and 14 dpf (n = 20) and conducted according to Colwill and Creton (2011) and Altenhofen et al. (2017). The experiments were performed in a temperature-controlled room (27 ± 2 °C) between 13:00 and 17:00. The exploratory behavior of the animal was recorded in a video for 5 min after the 1-min acclimatization (Colwill and Creton, 2011; Altenhofen et al., 2017). The performance was video recorded for automated analysis using EthoVision XT software (version 11.5, Noldus), which can track the swimming activity of the animals at a rate of 15 positions per second. The video-tracking data were then used to determine the relevant measures through detecting animals by looking at the contrast them and the background of the apparatus. Each larva was placed

individually in a 24-well cell culture plate that contained 2 mL water per well (Altenhofen et al., 2017; Altenhofen et al., 2019), in a designed protocol that virtually divided each 15 mm diameter well in the inside area (7.5 mm diameter) and outside area (7.5 mm diameter) (Altenhofen et al., 2019). The locomotor parameters evaluated were total distance traveled (m) and velocity (m/s, the ratio between distance traveled and movement) and were considered as parameters of exploration of the new environment. The parameter movement was defined as the period during which the zebrafish exceeded the start velocity (defined as 0.06 cm/s) and remained moving until reaching the stop velocity (defined as 0.01 cm/s; Mahmood et al., 2013). The anxiety-like behavior was also measured, and the time spent in each well position (outside vs. inside area) was considered an index of anxiety. This task exploits the natural tendency of zebrafish to spend most of the time in the outside area when introduced to a novel environment. Then, the animals gradually extend the swimming range to include the inside portion of the test well. A longer time spent in the outside area and less time spent in the inside of the well indicates increased anxiety (Colwill and Creton, 2011). We also determined the absolute turn angle (°) of the animal, which evaluates erratic movements (Nabinger et al., 2018). This measure reports the sum of the absolute angle between each movement vector of the animal. To calculate this parameter a vector of movement from one position of the animal's center point to the next is created. For each vector, the angle between it and the previous vector is calculated with anti-clockwise movement being negative and the clockwise movement is positive (i.e. the angle is from −180° to 180°). The absolute value of this angle is summed for all the positions of the animal throughout the test. The video recorded from the top of the well (Altenhofen et al., 2017).

2.4. Adults analysis

2.4.1. Body weight measurement

The individual body weight was calculated as the difference between baseline body weight, obtained before beginning 3-NPA treatment, and body weight at the end of treatment (Bortolatto et al., 2017):

Change in body weight = body weight 29th day − body weight 1st day.

2.4.2. Novel tank test

Twenty-four h after each injection (days 1, 5, 9, 13, 17, 21, and 25) and 120 h (day 29) after the last injection, the exploratory behavior of each animal was measured ($n = 20$). The experiments were performed in a temperature-controlled room (27 ± 2 °C) between 8:30 and 12:00. The animals were placed individually in experimental tanks (30 cm long × 15 cm high × 10 cm wide). After 60 s habituation, their locomotor behavior was recorded for 5 min (Altenhofen et al., 2017; Nabinger et al., 2018) for subsequent analysis with EthoVision XT software. The analyzed behavioral parameters were distance traveled (m), velocity (m/s, the ratio between distance traveled and movement), time spent in upper zone (s), and turn angle (°). The time spent in the upper zone is indicative of anxiolytic/depression-like behavior. When zebrafish are introduced into a new environment, they tend to spend more time at the bottom of the tank until gradually moving to the upper zone after a few minutes (Levin et al., 2007). The parameter movement was defined as the period during which the zebrafish exceeded the start velocity (0.6 cm/s) and remained moving until reaching the stop velocity (0.59 cm/s, Tran et al., 2016).

2.4.3. Social interaction

Zebrafish are schooling fish that may exhibit a preference for their conspecifics under certain circumstances. Adult social interaction was evaluated at the end of the 29-day treatment ($n = 20$) between 8:30 and 12:00. Each fish was individually placed in an experimental tank (30 cm long × 15 cm high × 10 cm wide). An empty fish tank was placed on one side of the experimental tank. The other side contained an identically sized tank that held 15 zebrafish, which were designated the "stimulus fish". The fish undergoing evaluation was allowed to acclimatize to the experimental tank for 30 s, after which its behavior was video recorded over 5 min for subsequent analysis with EthoVision XT, according to Nabinger et al. (2018). To quantify fish preference between the "stimulus fish" side of their tank at the expense of the empty tank, the experimental tank was virtually divided into four equal sections. Zones 1 and 2 corresponded to the segments closer to the conspecific school and zones 3 and 4 were considered as the segments closer to the empty tank. The amount of time the experimental fish spent in each zone was measured during the 5 min experiment.

2.4.4. Aggression test

After the social interaction analysis, aggressive behavior was evaluated at the end of the 29-day treatment in adult animals (n = 20) between 8:30 and 12:00. The mirror test was used to measure aggression according to the procedure described by Nabinger et al. (2018), with modifications. Each fish was individually placed in an experimental tank (30 cm long x 15 cm high x 10 cm wide). A mirror (45 cm × 38 cm) was placed at the side of the tank at an angle of 22.5° to the back wall of the tank so that the left vertical edge of the mirror touched the side of the tank and the right edge was further away. Thus, when the experimental fish swam to the left side of the tank, their mirror image appeared closer to them. After 1-min acclimatization, a 5-min session was recorded for subsequent quantification of aggression with EthoVision XT. Virtual vertical lines were used to divide the tank into four sections to allow the researchers to record the number of entries the fish made into each section. Entry to the left-most segment indicated a preference for proximity to the "opponent", whereas entry to the right-most segments implied avoidance. The amount of time the experimental fish spent in each segment was measured. We also evaluated the number of bites against the mirror image as an aggression parameter. The video-tracking data were used to determine the relevant measures through detecting animals by examining the contrast between them and the background of the apparatus.

2.4.5. Inhibitory avoidance task

To assess whether 3-NPA could impair memory in adult animals, we performed an inhibitory avoidance test at the end (29 days) of long-term treatment ($n = 10$) between 9:00 and 12:00 (Blank et al., 2009; Nabinger et al., 2018). There were two sessions, training and test, with a 24-h interval between them. In each session, animals were placed individually in an experimental tank (18 cm long × 9 cm wide × 7 cm high) with water, divided by a guillotine door into two compartments of equal size: one black (right side) and one white (left side). During the training session, the animal was placed in the white compartment (with the door closed) for 1-min habituation and environmental recognition. After this period, the divider was lifted. Once the animal crossed into the black side of the tank, the guillotine door was closed again, and two electrodes attached to an 8.8 V stimulator delivered a 3 ± 0.2 V AC shock pulse (intensity measured between electrodes and the center of the dark compartment) for 5 s. The animal was removed from the apparatus and returned to its housing tank with only water for 24 h until the test session, which consisted of the same protocol as the training session, but without the electric shock. The latency to enter the black compartment during each session was measured, and the expected increase in the test session was used as an index of memory retention. A 180-s ceiling was imposed on test session latency measurements.

2.5. Statistical analysis

The data are presented as the mean ± standard error of the mean (S.E.M), except for larval survival that is presented as percentages. Larval survival during the 14 experimental days was examined with Kaplan-Meier analysis. Data from heart rate, morphological evaluation, and locomotor parameters in larvae were evaluated with one-way

analysis of variance (ANOVA) followed by post-hoc comparisons using Tukey's test. Exploratory behavior data from adults were analyzed with two-way ANOVA followed by post-hoc comparisons using Bonferroni corrections. Body weight, social interaction and aggression data from adults were evaluated with one-way ANOVA followed by post-hoc comparisons using Tukey's test. Inhibitory avoidance training and test latencies for each group were compared using the Mann–Whitney *U* matched pairs test. The latencies of multiple groups were compared using Kruskal–Wallis and Mann–Whitney *U* tests. For all comparisons, the significance level was set at $p < 0.05$.

3. Results

3.1. 3-NPA exposure in zebrafish larvae

3.1.1. Survival

Exposure to 3-NPA decreased the larval survival rate when all groups were compared ($p < 0.0001$, $n = 50$). Larvae survival for the control group at the end of the experiment (14 dpf) was 96%. The survival rate decreased in animals exposed to 0.01 (68%, $p < 0.0004$), 0.05 (72%, $p < 0.0014$), 0.1 (74%, 0.0028), 0.2 (68%, $p < 0.0004$), and 0.5 (54%, $p < 0.0001$) mM 3-NPA. Moreover, animals exposed to 1.0 (0%, $p < 0.0001$) and 2.0 (0%, $p < 0.0001$) mM 3-NPA died by the seventh and fifth day of observation, respectively, with a survival percentage of 0%. Therefore, these concentrations were excluded from the study. Overall, all 3-NPA-exposed groups showed significant differences in survival from the control (96%) at the end of the experiment (14 days, Fig. 2).

3.1.2. Heart rate

Heart rate was measured at 2 dpf (Fig. 3a) and 5 dpf (Fig. 3b). At 2 dpf, embryos exposed to 0.2 and 0.5 mM 3-NPA exhibited an increased heart rate compared to unexposed controls, whereas at 5 dpf, larvae exposed to 0.1, 0.2, and 0.5 mM 3-NPA had an increased heart rate ($F_{(5,174)} = 4.051$; $p < 0.05$ at 2 dpf; $F_{(5,169)} = 4.255$; $p < 0.01$ at 5 dpf).

3.1.3. Morphology

The teratogenic effect of 3-NPA on the morphology of larvae was evaluated at 5 dpf. There were no differences in body length and eye surface area between the control and the 3-NPA exposed groups ($F_{(5,173)} = 1.589$; $p = 0.1656$; $F_{(5,172)} = 1.528$; $p = 0.1833$). However, there was a significant decrease in ocular distance with 0.05 mM 3-NPA treatment compared to the control group ($F_{(5,173)} = 4.224$; $p < 0.05$) (Table 1, Supplementary Fig. 1).

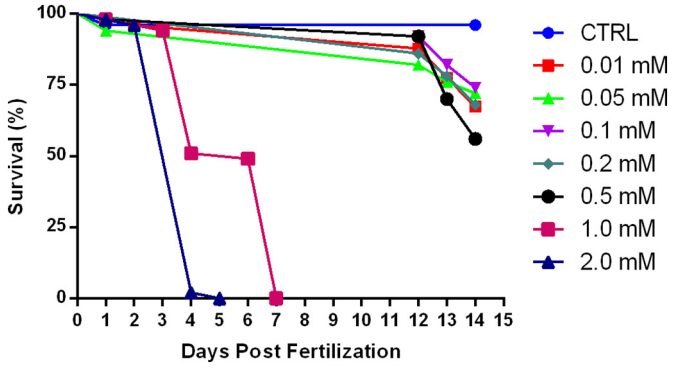

Fig. 2. Kaplan-Meir survival for zebrafish larvae treated with 3-nitropropionic acid. 3-Nitropropionic acid significantly impaired survival at 1.0 and 2.0 mM when compared to the control group at days 4, 5, and 7 of the experiment (Log-rank [Mantel-Cox] test, $p < 0.0001$). Additionally, there was a significant difference between the animals exposed to 0.01, 0.05, 0.1, 0.2, and 0.5 mM 3-nitropropionic acid and the control group (Log-rank [Mantel-Cox] test, $p < 0.05$). Data are expressed as the mean from 50 animals analyzed individually for each group.

3.1.4. Exploratory behavior

The exploratory behavior of the larvae was examined at 7, 10, and 14 dpf to determine whether 3-NPA exposure could alter larvae locomotion and orientation. There were no differences in distance traveled, velocity, time spent outside the area and absolute turn angle at any of these ages (Table 2, Supplementary Fig. 2).

3.2. 3-NPA treatment in adult zebrafish

3.2.1. Body weight

Administration of 3-NPA significantly altered adult body weight (Fig. 4). Specifically, 10 ($F_{(3,76)} = 4.474$; $p < 0.05$) and 60 ($F_{(3,76)} = 4.474$; $p < 0.01$) mg/kg 3-NPA significantly decreased body weight when compared to control animals. The control group showed an increase in body weight.

3.2.2. Novel tank test

The behavior pattern of adult animals was analyzed at days 1, 5, 9, 13, 17, 21, 25, and 29, approximately 24 h after each i.p. injection. All concentrations markedly altered the locomotion pattern of the exposed zebrafish compared to the controls (Fig. 5). The total distance traveled at 60 mg/kg 3-NPA was decreased at all time points (except day 9) when compared with the control group ($F_{(3,608)} = 56.24$; $p < 0.001$). In contrast, fish treated with 10 or 20 mg/kg 3-NPA demonstrated a difference in the distance traveled at days 1, 5, 9, 25, and 29 ($F_{(3,608)} = 56.24$; $p < 0.05$) and days 1, 13, and 25 ($F_{(3,608)} = 56.24$; $p < 0.05$), respectively (Fig. 5a). Furthermore, 60 mg/kg 3-NPA treatment decreased velocity only at day 25 when compared to the control group ($F_{(3,608)} = 8.073$; $p < 0.05$; Fig. 5b). To observe changes in anxiety, we evaluated the time the fish spent in the upper zone of the tank. As illustrated in Fig. 5c, there were no significant differences in the time spent in the upper zone among the 3-NPA treated groups at any time point when compared with the control group. Another analyzed locomotor parameter was the absolute turn angle. Zebrafish exposed to 20 or 60 mg/kg 3-NPA showed significant changes in the absolute turn angle at day 5 when compared to the control group ($F_{(3,605)} = 5.584$; $p < 0.05$; Fig. 5d, Table 3).

3.2.3. Aggression

Treatment with 3-NPA significantly impacted aggressive behavior, as evaluated by the time spent in the segment nearest to the mirror image. Our results showed that 60-mg/kg-treated animals spent less time in the segment nearest to the mirror compared to the control group ($F_{(3,76)} = 7.688$; $p < 0.01$; Fig. 6a). However, there were no differences at 10 or 20 mg/kg when compared with the control group. Moreover, the animals treated with 20 ($p < 0.05$; Fig. 6b) and 60 ($p < 0.01$; Fig. 6b) mg/kg 3-NPA showed a significant decrease in the biting episodes when compared to the control group.

3.2.4. Social interaction

Treatment with 3-NPA at all examined doses did not induce any social interaction deficit in zebrafish since there is no significant difference in the time spent in the stimulus zone between 3-NPA-treated and control animals (Fig. 6c). Also, 3-NPA treatment did not alter the mean distance traveled in the stimulus zone (Fig. 6d) and in the non-stimulus zone (Fig. 6e).

3.2.5. Inhibitory avoidance task

The effects of 3-NPA treatment on long-term memory were also analyzed in zebrafish. Sixty mg/kg 3-NPA treatment significantly impaired inhibitory avoidance long-term memory compared to the control group ($p < 0.05$; Fig. 7).

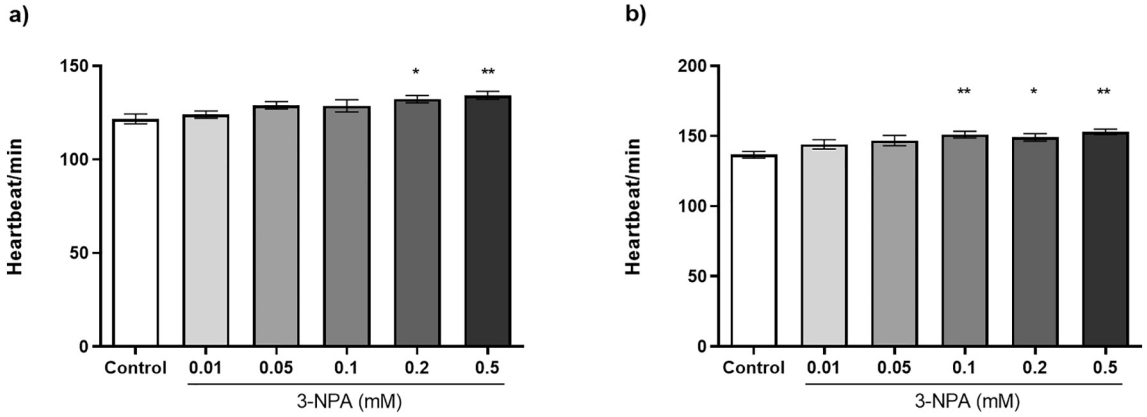

Fig. 3. Larval heart rate measured at 2 days post-fertilization (a) and 5 days post-fertilization (b). Data are expressed as the mean ± S.E.M (n = 30) and were analyzed with a one-way ANOVA followed by a post-hoc Tukey's test. * $p < 0.05$, ** $p < 0.01$.

Table 1
The effects of morphological parameters of control and 3-Nitropropionic acid-treated zebrafish larvae at 5 days post-fertilization.

Variable	Effects	Mean ± S.E.M (per group)	*F*-value	DF	*p*-Value
Body length (μm)	Treatment (Group)	Control: 3371 ± 23.05 0.01 mM 3-NPA: 3356 ± 18.50 0.05 mM 3-NPA: 3340 ± 14.23 0.1 mM 3-NPA: 3310 ± 22.97 0.2 mM 3-NPA: 3323 ± 16.74 0.5 mM 3-NPA: 3311 ± 22.41	1.589	5	0.1656
Ocular distance (μm)	Treatment (Group)	Control: 107.09 ± 2.064 0.01 mM 3-NPA: 97.69 ± 2.585 **0.05 mM 3-NPA: 95.37 ± 2.853** 0.1 mM 3-NPA: 100.5 ± 2.803 0.2 mM 3-NPA: 102.1 ± 2.331 0.5 mM 3-NPA: 109.1 ± 3.182	4.224	5	**0.0012**
Surface area (μm^2)	Treatment (Group)	Control: 48904 ± 823.3 0.01 mM 3-NPA: 50988 ± 1117 0.05 mM 3-NPA: 49533 ± 1052 0.1 mM 3-NPA: 46959 ± 1295 0.2 mM 3-NPA: 48517 ± 1090 0.5 mM 3-NPA: 47888 ± 1302	1.528	5	0.1833

Notes: DF, degrees of freedom; significant effects ($p < 0.05$) are given in bold font.

Table 2
Exploratory behavior of control and 3-NPA treated zebrafish larvae at 7, 10, and 14 days post-fertilization.

Variable	Effects	*F*-value	DF	*p*-Value
Distance total traveled (m) – 7 dpf	Treatment (Group)	1.381	5	0.2368
Distance total traveled (m) – 10 dpf	Treatment (Group)	0.7500	5	0.5877
Distance total traveled (m) – 14 dpf	Treatment (Group)	2.233	5	0.0557
Velocity (m/s) – 7 dpf	Treatment (Group)	1.932	5	0.0943
Velocity (m/s) – 10 dpf	Treatment (Group)	0.4962	5	0.7785
Velocity (m/s) – 14 dpf	Treatment (Group)	1.026	5	0.4058
Time spent outside area (s) – 7 dpf	Treatment (Group)	2.093	5	0.0713
Time spent outside area (s) – 10 dpf	Treatment (Group)	0.6331	5	0.6748
Time spent outside area (s) – 14 dpf	Treatment (Group)	0.2369	5	0.9455
Absolute turn angle (°) – 7 dpf	Treatment (Group)	1.030	5	0.4035
Absolute turn angle (°) – 10 dpf	Treatment (Group)	0.9006	5	0.4834
Absolute turn angle (°) – 14 dpf	Treatment (Group)	1.502	5	0.1947

Notes: DF, degrees of freedom; dpf, day post-fertilization; significant effects ($p < 0.05$) are given in bold font.

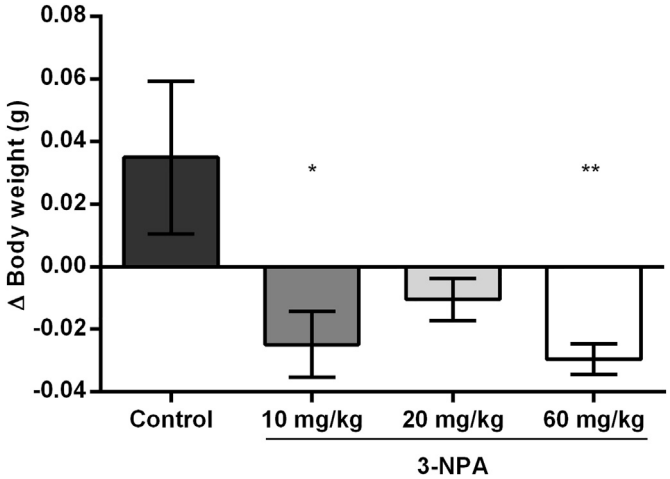

Fig. 4. Body weight of control and 3-nitropropionic acid-treated adult zebrafish. Weight measurements were performed before the first dose and at the end of the experiment. Data are expressed as the mean ± S.E.M (n = 20) and were analyzed with a one-way ANOVA followed by a post-hoc Tukey's test. * $p < 0.05$, ** $p < 0.01$.

4. Discussion

This study demonstrated that exposure to 3-NPA caused morphological and physiological alterations in zebrafish larvae. When the adult animals were exposed to 3-NPA, body weight, locomotor activity, and aggressive behavior were decreased, and there was impairment in long-term memory in the inhibitory avoidance task. To the best of our

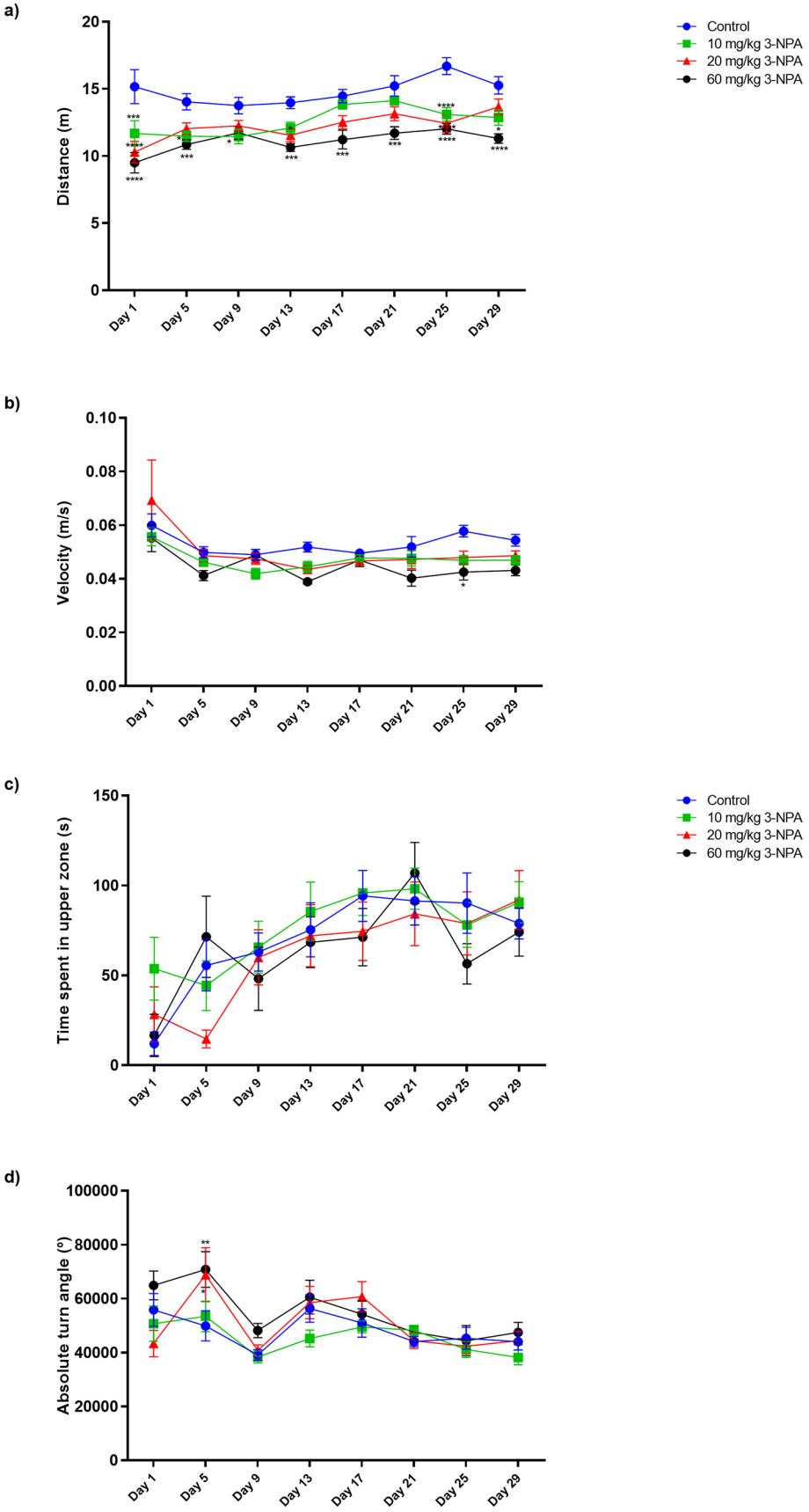

Fig. 5. Locomotion of control and 3-Nitropropionic acid-treated zebrafish. Twenty-four h after each injection, total distance traveled (a), velocity (b), time spent in the upper zone (c), and absolute turn angle (d) were evaluated. Data are expressed as the mean ± S.E.M (n = 20) and were analyzed with a two-way ANOVA followed by a Bonferroni post-hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 3
The main effects of behavior adult analysis and the interaction between day and group of treatment with 3-Nitropropionic acid.

Dependent variable	Effects	*F*-value	DF	*p*-Value
Total distance	Interaction	1.482	21	0.0768
	Day	6.508	7	**0.0001**
	Treatment (Group)	56.24	3	**0.0001**
Velocity	Interaction	0.9061	21	0.5834
	Day	6.995	7	**0.0001**
	Treatment (Group)	8.073	3	**0.0001**
Time spent in upper zone	Interaction	0.8515	21	0.6554
	Day	9.473	7	**0.0001**
	Treatment (Group)	1.431	3	0.2326
Absolute turn angle	Interaction	1.255	21	0.1987
	Day	9.132	7	**0.0001**
	Treatment (Group)	5.584	3	**0.0009**

Notes: DF, degrees of freedom; significant effects ($p < 0.05$) are given in bold font.

knowledge, this study is the first to demonstrate the toxic effects of 3-NPA in physiological and behavioral parameters in zebrafish larvae and adults.

Our findings showed that larvae exposed to the two highest 3-NPA concentrations had a mortality rate of 100% at 7 dpf. Studies reported that 3-NPA concentrations between 0.3 and 10 mM cause high cellular mortality and important biochemical changes (Ryu et al., 2003; Vis et al., 2004; Colle et al., 2018). Ryu et al. (2003) and Colle et al. (2012) observed in their in vitro studies that 3-NPA treatment at 1, 5, and 10 mM and 0.5 and 1 mM, respectively, reduced the viability and increased the cell death. In a recent study, Colle et al. (2018) observed that 0.3, 1, 3, and 10 mM 3-NPA significantly decrease viability in a culture of cortical neurons obtained from mouse and increase cell mortality. Vis et al. (2004) found that when cortical cells from mice are exposed to 50, 100, 250, or 500 μM 3-NPA for 24 h, there is elevated lactate dehydrogenase activity at 250 and 500 μM treatments and a dose-dependent increase in lactate and a decrease in pyruvate at all concentrations when compared to the control. These findings demonstrate the harmful potential of 3-NPA because concentrations lower than those used in our study can alter the biochemical machinery in cell culture.

Although HD is primarily a neurological disease in humans, it also affects other systems, including the cardiovascular system (Bellosta Diago et al., 2018). Cardiac alterations are observed in HD patients at the early and late stages of pathology (Kobal et al., 2017; Critchley et al., 2018). Previous studies with transgenic HD animal models corroborate the cardiac dysfunction in HD (Mihm et al., 2007; Kiriazis et al., 2012; Mielcarek et al., 2014). In the present study, larvae treated with 0.1, 0.2, and 0.5 mM 3-NPA showed an increased heart rate at 2 and 5 dpf. Moreira (2017), when examining the effects of 3-NPA on intrinsic innervation of the heart in mice, found an increase in heart rate when compared to the control group. The mechanisms that underlie 3-NPA cardiotoxicity can involve inhibition of oxygen consumption and reduction of ATP, oxidative damage caused by increased oxidized proteins, lipid peroxidation, and the consequent generation of reactive oxygen species in cardiac cells (Castillo et al., 1993; Lopez et al., 1998; Milutinović and Zorc-Plesković, 2012; Silva-Palacios et al., 2017). These mechanisms can promote cardiac insufficiency and decrease cardiac output, changes that will reduce the oxygen supply and blood flow below the levels needed for metabolic demands. Consequently, the sympathetic activity will increase to promote cardiac vasoconstriction and elevate the heart rate.

Importantly, we also observed that 3-NPA causes small morphological alterations in zebrafish larvae. The findings showed that 0.05 mM 3-NPA decreased the ocular distance when analyzed at 5 dpf. However, this change may have limited biological significance, given the subtle effect and lack of a dose-response relationship. Furthermore, this mycotoxin did not alter other morphological parameters such as body length and the surface area of the eyes. Lumsden et al. (2007) and Diekmann et al. (2009) observed that huntingtin-deficient zebrafish larvae have remarkably smaller head and eyes compared to the control fish. Studies also revealed that 3-NPA-like mycotoxins may cause morphological changes in zebrafish larvae. Zebrafish exposure to the mycotoxin ochratoxin A (OTA) decreases the growth of the animal and causes craniofacial deformity and curvatures in the body (Haq et al., 2016; Khezri et al., 2018).

Toxins, such as paraquat and mitoparaquat, that affect the mitochondrial complex may alter exploratory parameters in zebrafish larvae (Wang et al., 2018; Pinho et al., 2019). In contrast to these studies, we demonstrated that 3-NPA exposure did not alter locomotion in zebrafish larvae. Kotlar et al. (2018) also observed that 1 and 10 mM 3-NPA is unable to alter locomotion in animals when comparing the toxic effects of quinolinic acid and 3-NPA in the nematode *Caenorhabditis elegans*. 3-NPA is a toxin that affects energy metabolism, and thus it is expected to affect the locomotor activity of zebrafish larvae. The unexpected lack of a 3-NPA effect on locomotor parameters may be due to adaptive responses to energy decrease occurring at this stage of development.

Weight loss occurs in HD patients and is a hallmark of the disease (Chen, 2011; van der Burg et al., 2017). Interestingly, in our study, animals administered 10 and 60 mg/kg 3-NPA showed weight reduction compared to control animals. According to the literature, 3-NPA decreases body weight due to interference with energy metabolism (Saydoff et al., 2003; Ahuja et al., 2008). Our results are consistent with the literature since previous studies in rodents showed that 3-NPA treatment induces weight loss (Saydoff et al., 2003; Ahuja et al., 2008; Dhadde et al., 2016; Bortolatto et al., 2017).

During the manifestation phase of HD in humans, hyperkinesia is observed during the initial stage and bradykinesia at the late stage (Ghosh and Tabrizi, 2018). He et al. (1995) and Ming (1995) were the first to report that human intoxication with 3-NPA (through the ingestion of sugarcane) causes an acute non-inflammatory syndrome, with development of motor symptoms similar to those of HD, such as choreatic movements in the hand and fingers, facial grimace, dysarthria, and dystonia. 3-NPA toxicity in animals was first seen in Western regions of North America when animals poisoned with *Indigofera* and *Astragallus* plants had motor abnormalities such as muscle weakness, motor incoordination, and rigidity (Ludolph et al., 1991). When injected with 3-NPA, different animal species can mimic HD symptoms. However, sensitivity to this toxin differs according to the species. Doses should be chosen and administered according to the phenotypic profile and desired biochemical changes (Túnez and Santamaría, 2009).

In adult zebrafish, our data demonstrated that 10 and 20 mg/kg 3-NPA treatment reduced locomotion, but not at every evaluation. However, treatment with 60 mg/kg reduced locomotion from the first injection (1 day) until the end of the treatment (i.e., after the last i.p. injection on day 29). Thus, while there was a pattern of decreased locomotor activity for all doses, 60 mg/kg 3-NPA would be the most effective and best dose to study the late-stage of HD hypolocomotion from to start until the end of the treatment. Studies in rodents (rats) demonstrated that acute treatment with 3-NPA (two 10-mg/kg doses) induces hyperkinetic locomotive phenotypes (Hamilton and Gould, 1987; Borlongan et al., 1997). Chronic 3-NPA administration (more than four injections over a course of days) causes hypokinetic movements in non-human primates (Brouillet et al., 1995) and rats (Borlongan et al., 1997; Guyot et al., 1997). This hypokinetic pattern is hypothesized to result from a decrease in dopamine and its metabolites because this neurotransmitter is responsible for motor, cognitive, and memory functions (Arias-Carrión et al., 2010; Cepeda et al., 2014; Kacsprzak et al., 2017).

We also measured the absolute turn angle, which reveals changes in the swimming direction of zebrafish. The groups treated with 20 and 60 mg/kg 3-NPA demonstrated an increased turning angle only at day

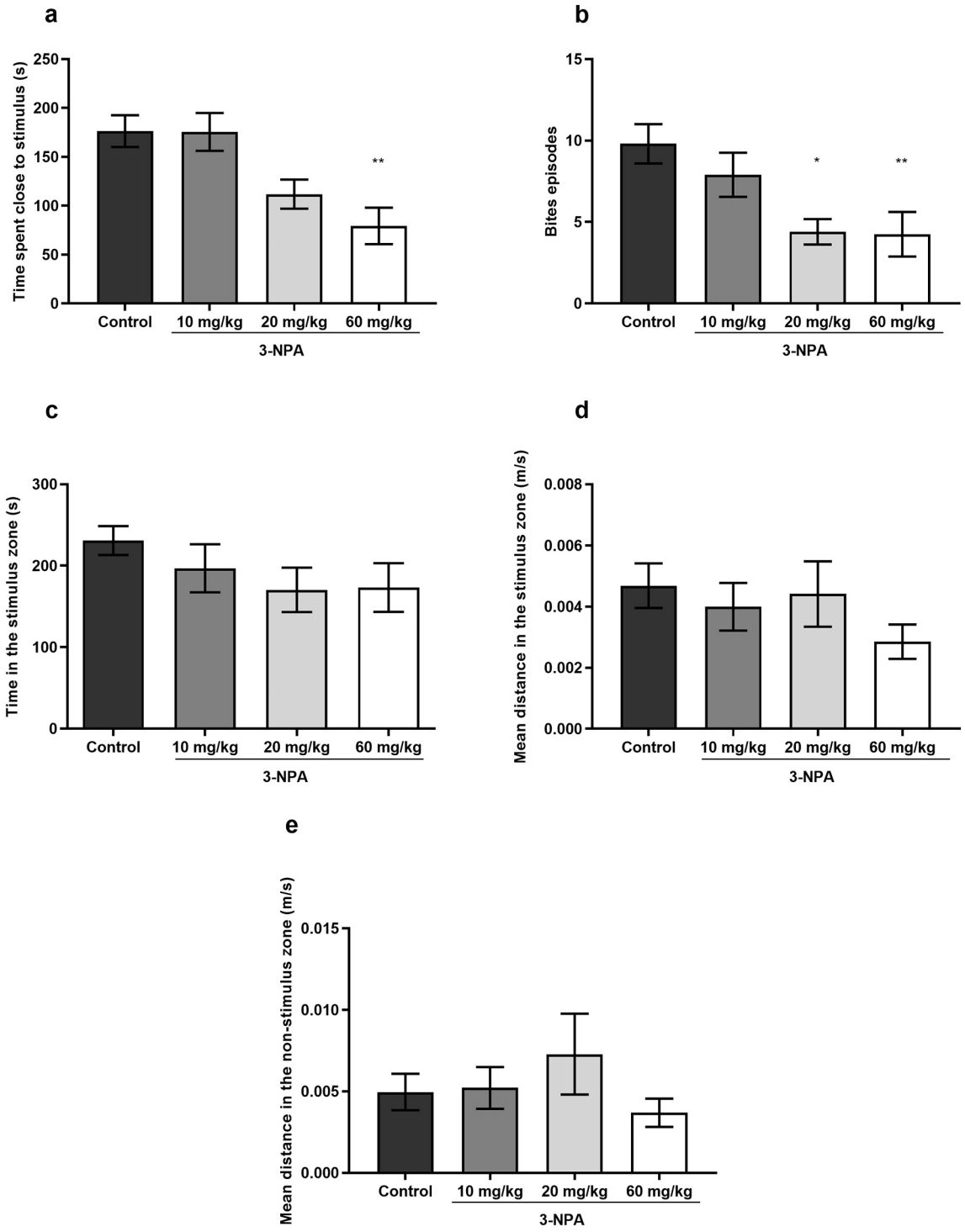

Fig. 6. Effects of 3-Nitropropionic acid on aggression (aand b) and social interaction (c, d and e) in zebrafish. Time spent close to stimulus in mirror (a), bite episodes (b), time spent in stimulus zone (c), mean distance in the stimulus zone (d) and mean distance in the non-stimulus zone (e). Data are expressed as the mean ± S.E.M (n = 20) and were analyzed with a one-way ANOVA followed by a post-hoc Tukey's test.

5. Consistently, Bortolotto et al. (2014), when analyzing the behavioral parameters in adult zebrafish treated with i.p. injections of paraquat, an herbicide that reproduces the symptoms of Parkinson's disease, showed that the 10 mg/kg body weight paraquat-treated group exhibits an increase in the turning angle only at day 10. In addition to motor alterations that result from the degeneration of dopaminergic neurons, HD causes non-motor symptoms. Multiple neuronal systems appear to be involved in HD pathophysiology, and these dysfunctions lead to cognitive and psychiatric disturbances (Glass et al., 2000; Cepeda et al., 2014; Ferrante et al., 2014). Therefore, we also evaluated cognitive and social behaviors in adult zebrafish treated with 3-NPA, such as anxiety, social interaction, aggression, and memory.

When introduced into a new environment, the zebrafish stayed at the bottom of the aquarium and gradually explored the upper portions. We did not observe any changes in the time spent in the upper zone between the 3-NPA-treated and control animals. These data suggest that treatment with 3-NPA does not cause anxiety-like behavior in these animals. These results conflict with a previous study conducted in

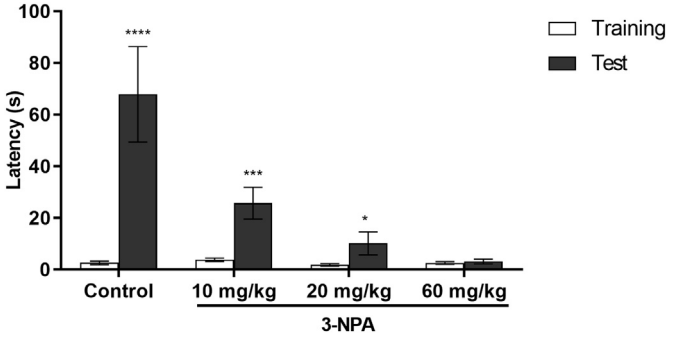

Fig. 7. Inhibitory avoidance task performance for training and long-term memory test sessions of control and 3-Nitropropionic acid-treated adult zebrafish after 28 days of treatment. Data are expressed as the mean ± S.E.M ($n = 10$) and were analyzed individually for each group; * $p < 0.05$, *** $p < 0.001$, and **** $p < 0.0001$ indicate the differences between training and test sessions for each group using the Mann-Whitney *U* matched pair test. There were no differences between training performances among all groups, as evaluated by the Kruskal-Wallis test.

Wistar rats, where 10 mg/kg 3-NPA injections for 14 days promote anxiety-like behavior (Jain and Gangshettiwar, 2014). Previous studies that addressed anxiety-like behavior in a transgenic rat model of HD at different ages observed that the animals at 6, 8, 10, 12, 15, and 20 weeks have reduced anxiety-like behavior when compared to wild type littermates (File et al., 1998; Cao et al., 2006; Zeef et al., 2012). These divergent results may be due to age, treatment time, and variability among animal species.

Apathy and aggression are two neuropsychiatric alterations of HD. The genetic rodent HD model exhibits alteration in social interaction and aggressive behavior when compared to animals without this pathology (Shelbourne et al., 1999; Wood and Morton, 2015; Manfré et al., 2018). Zebrafish exhibit social behavior, including aggression and social interaction, and they greatly prefer their conspecifics under certain circumstances (Das and Rajanikant, 2014). In the present study, we analyzed whether treatment with 3-NPA altered this conspecific preference. We observed that treated animals did not differ from their controls. However, 60 mg/kg 3-NPA treatment decreased aggressive behavior during the mirror image test. Moreover, at 20 and 60 mg/kg 3-NPA, there was a decrease in the number of bite episodes against the mirror image. This decrease in aggression may be related to the reduced locomotion of these animals after chronic 3-NPA exposure.

We also evaluated long-term memory using the inhibitory avoidance task. We found a significant impairment in long-term memory in 60 mg/kg 3-NPA-treated animals. These data suggest that long-term exposure to 3-NPA induces memory impairment. Studies demonstrated that HD patients present cognitive decline at various stages of the disease. These deficits are observed in the subject's attention, psychomotor speed, executive functions, learning, and short and long-term memory, but long-term memory is impaired only at the final stage of the pathology (Lawrence et al., 1998; Paulsen et al., 2001; Beglinger et al., 2005). Kamble et al. (2018), when analyzing changes in cortical excitability and cognitive dysfunction in HD through neuropsychological tests, demonstrated that HD patients have significant impairment in these measures when compared to subjects without the disease. These findings suggest that attention, memory, learning, visuospatial abilities, and cognitive functions are impaired in HD (Kamble et al., 2018). Previous studies revealed that 3-NPA treatment in non-human primates and rats impairs cognitive and memory tasks (Palfi et al., 1996; Kumar et al., 2010). Therefore, our results are consistent with the literature and further suggest that 3-NPA induces memory impairment.

In summary, our findings indicate that 3-NPA exposure during the early stages of life induces morphological and cardiac physiological alterations. Furthermore, in adult zebrafish, repeated i.p. injections of 3-NPA produced a hypolocomotion profile, impaired long-term memory, decreased aggressive behavior, and promoted weight loss. Therefore, we identified symptoms observed in the late stage of HD through long-term exposure to 3-NPA in zebrafish. This study is the first to report 3-NPA-mediated neurotoxic alterations in zebrafish that closely resemble the behavioral pathology of late-stage HD. Future studies could use a zebrafish model to screen drugs for their potential to ameliorate late-stage HD symptoms. Taken together, these results highlight the importance of zebrafish as a complementary and alternative model for mammals to study the mechanisms and the behavioral phenotype of HD.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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; 305035/2015-0), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS; 17/2551-0000977-0), and Instituto Nacional de Ciência e Tecnologia para Doenças Cerebrais, Excitotoxicidade e Neuroproteção.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpc.2020.108772.

References

Ahuja, M., Bishnoi, M., Chopra, K., 2008. Protective effect of minocycline, a semi-synthetic second-generation tetracycline against 3-nitropropionic acid (3-NP)-induced neurotoxicity. Toxicology 244, 111–122.
Altenhofen, S., Wiprich, M.T., Nery, L.R., Leite, C.E., Vianna, M.R.M.R., Bonan, C.D., 2017. Manganese(II) chloride alters behavioral and neurochemical parameters in larvae and adult zebrafish. Aquat. Toxicol. 182, 172–183.
Altenhofen, S., Nabinger, D.D., Bitencourt, P.E.R., Bonan, C.D., 2019. Dichlorvos alters morphology and behavior in zebrafish (Danio rerio) larvae. Environ. Pollut. 245, 1117–1123.
Arias-carrión, O., Stamelou, M., Murillo-rodríguez, E., Menéndez-González, M., Pöppel, E., 2010. Dopaminergic reward system: a short integrative review. Int. Arch. Med. 6, 3–24.
Baig, S.S., Strong, M., Quarrell, O.W., 2016. The global prevalence of Huntington's disease: a systematic review and discussion. Neurodegener. Dis Manag. 6, 331–343.
Bailus, B., Zhang, N., Ellerby, L.M., 2017. Using genome engineering to understand Huntington's disease. In: Jaenisch, R., Zhang, F., Gage, F. (Eds.), Genome Editing in Neurosciences. Springer.
Baxendale, S., van Eeden, F., Wilkinson, R., 2017. The power of zebrafish in personalised medicine. Adv. Exp. Med. Biol. 1007, 179–197.
Beglinger, L.J., Nopoulos, P.C., Jorge, R.E., Langbehn, D.R., Mikos, A.E., Moser, D.J., Duff, K., Robinson, R.G., Paulsen, J.S., 2005. White matter volume and cognitive dysfunction in early Huntington's disease. Cogn. Behav. Neurol. 18, 102–107.
Bellosta Diago, E., Pérez-Pérez, J., Santos Lasaosa, S., Viloria Alebesque, A., Martínez-Horta, S., Kulisevsky, J., López del Val, J., 2018. Neurocardiovascular pathology in pre-manifest and early-stage Huntington's disease. Eur. J. Neurol. 25, 956–962.
Blank, M., Guerim, L.D., Cordeiro, R.F., Vianna, M.R.M., 2009. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. Neurobiol. Learn. Mem. 92, 529–534.
Blum, D., Chern, Y., Domenici, M.R., Buée, L., Lin, C.-Y., Rea, W., Ferré, S., Popoli, P., 2018. The role of adenosine tone and adenosine receptors in Huntington's disease. J. Caffeine Adenosine Res. 8, 43–58.
Borlongan, C.V., Koutouzis, T.K., Randall, T.S., Freeman, T.B., Cahill, D.W., Sanberg, P.R., 1995. Systemic 3-nitropropionic acid: behavioral deficits and striatal damage in adult rats. Brain Res. Bull. 36, 549–556.
Borlongan, C.V., Koutouzis, T.K., Freeman, T.B., Hauser, R.A., Cahill, D.W., Sanberg, P.R., 1997. Hyperactivity and hypoactivity in a rat model of Huntington's disease: the systemic 3-nitropropionic acid model. Brain Res. Protocol. 1, 253–257.
Bortolatto, C.F., Reis, A.S., Pinz, M.P., Voss, G.T., Oliveira, R.L., Vogt, A.G., Roman, S., Jesse, C.R., Luchese, C., Wilhelm, E.A., 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.
Bortolotto, J.W., Cognato, G.P., Christoff, R.R., Roesler, L.N., Leite, C.E., Kist, L.W., Bogo, M.R., Vianna, M.R., Bonan, C.D., 2014. Long-term exposure to Paraquat alters behavioral parameters and dopamine levels in adult Zebrafish (*Danio Rerio*). Zebrafish 11, 142–153.
Brouillet, E., Hantraye, P., Ferrante, R.J., Dolan, R., Leroy-Willig, A., Kowall, N.W., Beal, M.F., 1995. Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. Proc. Natl. Acad. Sci. 92, 7105–7109.
Brouillet, E., Condé, F., Beal, M.F., Hantraye, P., 1999. Replicating Huntington's disease phenotype in experimental animals. Prog. Neurobiol. 59, 427–468.
Cao, C., Teme, Y., Blokland, A., Ozen, H., Steinbusch, H.W.M., Vlamings, R., Nguyen, H.P., von Hörsten, S., Schmitz, C., Visser-Vandewalle, V., 2006. Progressive deterioration of reaction time performance and choreiform symptoms in a new Huntington's disease transgenic rat model. Behav. Brain Res. 170, 257–261.
Carmo, C., Naia, L., Lopes, C., Rego, A.C., 2018. Mitochondrial dysfunction in Huntington's disease. Adv. Exp. Med. Biol. 1049, 59–83.
Castillo, C., Valencia, I., Reyes, G., Hong, E., 1993. 3-Nitropropionic acid, obtained from astragalus species, has vasodilator and antihypertensive properties. Drug Dev. Res. 28, 183–188.
Cepeda, C., Murphy, K.P.S., Parent, M., Levine, M.S., 2014. The role of dopamine in huntington's disease. Prog. Brain Res. 211, 235–254.
Chan, A.W., Jiang, J., Chen, Y., Prucha, M.S., Hu, Y., Chi, T., Moran, S., Rahim, T., Li, S., Li, X., Zola, S.M., Testa, C.M., Mao, H., Villalba, R., Smith, Y., Zhang, X., Bachevalier, J., 2015. Progressive cognitive deficit, motor impairment and striatal pathology in a transgenic Huntington disease monkey model from infancy to adulthood. PLoS One 12, e0122335.
Chen, C.M., 2011. Mitochondrial dysfunction, metabolic deficits, and increased oxidative stress in Huntington's disease. Chang Gung Med. J. 34, 135–152.
Colle, D., Hartwig, J.M., Soares, F.A., Farina, M., 2012. Probucol modulates oxidative stress and excitotoxicity in Huntington's disease models in vitro. Brain Res. Bull. 10, 397–405.
Colle, D., Santos, D.B., de Souza, V., Lopes, M.W., Leal, R.B., de Souza Brocardo, P., Farina, M., 2018. Sodium selenite protects from 3-nitropropionic acid-induced oxidative stress in cultured primary cortical neurons. Mol. Biol. Rep. 46 (1), 751–762.
Colwill, R.M., Creton, R., 2011. Locomotor behaviors in zebrafish (Danio rerio) larvae. Behav. Process. 86, 222–229.
Coppen, E.M., Roos, R.A.C., 2017. Current pharmacological approaches to reduce chorea in Huntington's disease. Drugs 77, 29–46.
Critchley, B.J., Isalan, M., Mielcarek, M., 2018. Neuro-cardio mechanisms in Huntington's disease and other neurodegenerative disorders. Front. Physiol. 9, 1–8.
Croce, K.R., Yamamoto, A., 2018. A role for autophagy in Huntington's disease. Neurobiol. Dis. 0–1.
Da Silva, R.B., Siebel, A.M., Bonan, C.D., 2015. The role of purinergic and dopamineric systems on MK-801- induced antidepressant effects in zebrafish. Pharmacol. Biochem. Behav. 139, 149–157.
d'Amora, M., Giordani, S., 2018. The utility of zebrafish as a model for screening developmental neurotoxicity. Front. Neurosci. 12, 976.
Das, S., Rajanikant, G.K., 2014. Huntington disease: can a zebrafish trail leave more than a ripple? Neurosci. Biobehav. Rev. 45, 258–261.
Dhadde, S.B., Nagakannan, P., Roopesh, M., Anand Kumar, S.R., Thippeswamy, B.S., Veerapur, V.P., Badami, S., 2016. Effect of embelin against 3-nitropropionic acid-induced Huntington's disease in rats. Biomed. Pharmacother. 77, 52–58.
Diekmann, H., Anichtchik, O., Fleming, A., Futter, M., Goldsmith, P., Roach, A., Rubinsztein, D.C., 2009. Decreased BDNF levels are a major contributor to the embryonic phenotype of Huntingtin knockdown Zebrafish. J. Neurosci. 29, 1343–1349.
Dufour, B.D., McBride, J.L., 2019. Normalizing glucocorticoid levels attenuates metabolic and neuropathological symptoms in the R6/2 mouse model of huntington's disease. Neurobiol. Dis. 121, 214–229.
Fernagut, P.O., Diguet, E., Stefanova, N., Biran, M., Wenning, G.K., Canioni, P., Bioulac, B., Tison, F., 2002. Subacute systemic 3-nitropropionic acid intoxication induces a distinct motor disorder in adult C57Bl/6 mice: Behavioural and histopathological characterisation. Neuroscience 114, 1005–1017.
Ferrante, A., Martire, A., Pepponi, R., Varani, K., Vincenzi, F., Ferraro, L., Beggiato, S., Tebano, M.T., Popoli, P., 2014. Expression, pharmacology and functional activity of adenosine A1 receptors in genetic models of Huntington's disease. Neurobiol. Dis. 71, 193–204.
File, S.E., Mahal, A., Mangiarini, L., Bate, G.P., 1998. Striking changes in anxiety in Huntington's disease transgenic mice. Brain Res. 805, 234–240.
Fleming, A., Diekmann, H., Goldsmith, P., 2013. Functional characterisation of the maturation of the blood-brain barrier in larval zebrafish. PLoS One 10, 77548.
Fontana, B.D., Mezzomo, N.J., Kalueff, A.V., Rosemberg, D.B., 2018. The developing utility of zebrafish models of neurological and neuropsychiatric disorders: a critical review. Exp. Neurol. 299, 157–171.
Ghosh, R., Tabrizi, S.J., 2018. Clinical features of Huntington's disease. Adv. Exp. Med. Biol. 1049, 1–28.
Glass, M., Dragunow, M., Faull, R.L.M., 2000. The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(a) receptor alterations in the human basal ganglia in Huntington's disease. Neuroscience 97, 505–519.
Goldsmith, P., 2004. Zebrafish as a pharmacological tool: the how, why and when. Curr. Opin. Pharmacol. 4, 504–512.
Gu, M., Gash, M.T., Mann, V.M., Javoy-Agid, F., Cooper, J.M., Schapira, A.H.V., 1996. Mitochondrial defect in Huntington's disease caudate nucleus. Ann. Neurol. 39, 385–389.
Guyot, M.C., Hantraye, P., Dolan, R., Palfi, S., Maziére, M., Brouillet, E., 1997. Quantifiable bradykinesia, gait abnormalities and Huntington's disease- like striatal lesions in rats chronically treated with 3-nitropropionic acid. Neuroscience 79, 45–56.
Hamilton, B.F., Gould, D.H., 1987. Nature and distribution of brain lesions in rats intoxicated with 3-nitropropionic acid: a type of hypoxic (energy deficient) brain damage. Acta Neuropathol. 72, 286–297.
Haq, M., Gonzalez, N., Mintz, K., Jaja-Chimedza, A., De Jesus, C.L., Lydon, C., Welch, A., Berry, J.P., 2016. Teratogenicity of ochratoxin a and the degradation product, ochratoxin α, in the zebrafish (Danio rerio) embryo model of vertebrate development. Toxins (Basel) 8, 8–11.
He, F., Zhang, S., Qian, F., Zhang, C., 1995. Delayed dystonia with striatal ct lucencies induced by a mycotoxin (3-nitropropionic acid). Neurology 45, 2178–2183.
Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J.C., Koch, R., Rauch, G.J., White, S., Chow, W., Kilian, B., Quintais, L.T., Guerra-Assunção, J.A., Zhou, Y., Gu, Y., Yen, J., Vogel, J.H., Eyre, T., Redmond, S., Banerjee, R., Chi, J., Fu, B., Langley, E., Maguire, S.F., Laird, G.K., Lloyd, D., Kenyon, E., Donaldson, S., Sehra, H., Almeida-King, J., Loveland, J., Trevanion, S., Jones, M., Quail, M., Willey, D., Hunt, A., Burton, J., Sims, S., McLay, K., Plumb, B., Davis, J., Clee, C., Oliver, K., Clark, R., Riddle, C., Eliott, D., Threadgold, G., Harden, G., Ware, D., Mortimer, B., Kerry, G., Heath, P., Phillimore, B., Tracey, A., Corby, N., Dunn, M., Johnson, C., Wood, J., Clark, S., Pelan, S., Griffiths, G., Smith, M., Glithero, R., Howden, P., Barker, N., Stevens, C., Harley, J., Holt, K., Panagiotidis, G., Lovell, J., Beasley, H., Henderson, C., Gordon, D., Auger, K., Wright, D., Collins, J., Raisen, C., Dyer, L., Leung, K., Robertson, L., Ambridge, K., Leongamornlert, D., McGuire, S., Gilderthorp, R., Griffiths, C., Manthravadi, D., Nichol, S., Barker, G., Whitehead, S., Kay, M., Brown, J., Murnane, C., Gray, E., Humphries, M., Sycamore, N., Barker, D., Saunders, D., Wallis, J., Babbage, A., Hammond, S., Mashreghi-Mohammadi, M., Barr, L., Martin, S., Wray, P., Ellington, A., Matthews, N., Ellwood, M., Woodmansey, R., Clark, G., Cooper, J., Tromans, A., Grafham, D., Skuce, C., Pandian, R., Andrews, R., Harrison, E., Kimberley, A., Garnett, J., Fosker, N., Hall, R., Garner, P., Kelly, D., Bird, C., Palmer, S., Gehring, I., Berger, A., Dooley, C.M., Ersan-Ürün, Z., Eser, C., Geiger, H., Geisler, M., Karotki, L., Kirn, A., Konantz, J., Konantz, M., Oberländer, M., Rudolph-Geiger, S., Teucke, M., Osoegawa, K., Zhu, B., Rapp, A., Widaa, S., Langford, C., Yang, F., Carter, N.P., Harrow, J., Ning, Z., Herrero, J., Searle, S.M.J., Enright, A., Geisler, R., Plasterk, R.H.A., Lee, C., Westerfield, M., De Jong, P.J., Zon, L.I., Postlethwait, J.H., Nüsslein-Volhard, C., Hubbard, T.J.P., Crollius, H.R., Rogers, J., Stemple, D.L., 2013. The zebrafish reference genome sequence and its relationship to the human genome. Nature 496, 498–503.
Illarioshkin, S.N., Klyushnikov, S.A., Vigont, V.A., Kaznacheyeva, E.V., 2018. Molecular pathogenesis in Huntington's disease. Bichemistry (Mosc) 83, 1030–1039.
Jain, D., Gangshettiwar, A., 2014. Combination of lycopene, quercetin and poloxamer188 alleviates anxiety and depression in 3-nitropropionic acid-induced Huntington's disease in rats. J Intercult Ethnopharmacol 3, 186.
Johri, A., Chandra, A., Beal, M.F., 2013. PGC-1α, mitochondrial dysfunction, and Huntington's disease. Free Radic. Biol. Med. 62, 37–46.
Kacsprzak, V., Patel, N., Riley, E., Yu, L., Yeh, J.-R.J., Zhdanova, I.V., 2017. Dopaminergic control of anxiety in young and aged zebrafish. Pharmacol. Biochem. Behav. 157, 1–8.
Kamble, N., Netravathi, M., Nagaraju, B.C., Lenka, A., Kumar, K., Sowmya, V., Jain, S., Pal, P.K., 2018. Evaluation of cognition and cortical excitability in Huntington's disease. Can J Neurol Sci 45, 176–181.
Kaur, N., Jamwal, S., Kaur Gill, H., Bansal, P.K., 2017. Animal models of Huntington's disease. In: Bansal, P., Deshmukh, R. (Eds.), Animal Models of Neurological Disorders. Springer, Singapore.
Khezri, A., Herranz-Jusdado, J.G., Ropstad, E., Fraser, T.W., 2018. Mycotoxins induce developmental toxicity and behavioural aberrations in zebrafish larvae. Environ. Pollut. 242, 500–506.
Kim, G.W., Copin, J.C., Kawase, M., Chen, S.F., Sato, S., Gobbel, G.T., Chan, P.H., 2000. Excitotoxicity is required for induction of oxidative stress and apoptosis in mouse striatum by the mitochondrial toxin, 3-nitropropionic acid. J. Cereb. Blood Flow Metab. 20, 119–129.
Kiriazis, H., Jennings, N.L., Davern, P., Lambert, G., Su, Y., Pang, T., Du, X., La Greca, L., Head, G.A., Hannan, A.J., Du, X.J., 2012. Neurocardiac dysregulation and neurogenic arrhythmias in a transgenic mouse model of Huntington's disease. J. Physiol. 590, 5845–5860.
Kobal, J., Cankar, K., Pretnar, J., Zaletel, M., Kobal, L., Teran, N., Melik, Z., 2017. Functional impairment of precerebral arteries in Huntington disease. J. Neurol. Sci. 372, 363–368.
Kotlar, I., Colonnello, A., Aguilera-González, M.F., Avila, D.S., de Lima, M.E., García-Contreras, R., Ortíz-Plata, A., Soares, F.A.A., Aschner, M., Santamaría, A., 2018. Comparison of the toxic effects of quinolinic acid and 3-nitropropionic acid in *C. elegans*: involvement of the SKN-1 pathway. Neurotox. Res. 33, 259–267.
Kumar, P., Kalonia, H., Kumar, A., 2010. Nitric oxide mechanism in the protective effect of antidepressants against 3-nitropropionic acid-induced cognitive deficit, glutathione and mitochondrial alterations in animal model of Huntington's disease. Behav. Pharmacol. 21, 217–230.
Lawrence, A.D., Sahakian, B.J., Robbins, T.W., 1998. Cognitive functions and corticostriatal circuits: insights from Huntington's disease. Trends Cogn. Sci. 1, 379–388.
Levin, E.D., Bencan, Z., Cerutti, D.T., 2007. Anxiolytic effects of nicotine in zebrafish. Physiol. Behav. 90, 54–58.
Lopez, P.S., Castillo, C.H., Pastelín, G.H., Hernández, M.R., Suárez, M.J., Sánchez, M.L., Escalante, B.A., 1998. Characterization of 3-nitropropionic acid-induced bradycardia in isolated atria. Toxicol. Appl. Pharmacol. 148, 1–6.
Ludolph, A.C., He, F., Spencer, P.S., Hammerstad, J., Sabri, M., 1991. 3-nitropropionic

acid — exogenous animal neurotoxin and possible human striatal toxin. Can J Neurol Sci 18, 492–498.
Lumsden, A.L., Henshall, T.L., Dayan, S., Lardelli, M.T., Richards, R.I., 2007. Huntingtin-deficient zebrafish exhibit defects in iron utilization and development. Hum. Mol. Genet. 16, 1905–1920.
Lutte, A.H., Capiotti, K.M., Silva, N.L.G. da, Silva, C.S. de O. da, Kist, L.W., Bogo, M.R., Silva, R.S. Da, 2015. Contributions from extracellular sources of adenosine to the ethanol toxicity in zebrafish larvae. Reprod. Toxicol. 53, 82–91.
Macchi, F.S., Pissinate, K., Villela, A.D., Abbadi, B.L., Rodrigues-Junior, V., Nabinger, D.D., Altenhofen, S., Sperotto, N., da Silva Dadda, A., Subtil, F.T., de Freitas, T.F., Erhart Rauber, A.P., Borsoi, A.F., Bonan, C.D., Bizarro, C.V., Basso, L.A., Santos, D.S., Machado, P., 2018. 1-H-Benzo[d]imidazoles and 3,4-dihydroquinazolin-4-ones: design, synthesis and antitubercular activity. Eur. J. Med. Chem. 155, 153–164.
Mahmood, F., Fu, S., Cooke, J., Wilson, S.W., Cooper, J.D., Russell, C., 2013. A zebrafish model of CLN2 disease is deficient in tripeptidyl peptidase 1 and displays progressive neurodegeneration accompanied by a reduction in proliferation. Brain 136, 1488–1507.
Manfré, G., Novati, A., Faccini, I., Rossetti, A.C., Bosch, K., Molteni, R., Riva, M.A., Van der Harst, J.E., Nguyen, H.P., Homberg, J.R., 2018. BACHD rats expressing full-length mutant huntingtin exhibit differences in social behavior compared to wild-type littermates. PLoS One 13, 1–18.
Mason, S.L., Daws, R.E., Soreq, E., Johnson, E.B., Scahill, R.I., Tabrizi, S.J., Barker, R.A., Hampshire, A., 2018. Predicting clinical diagnosis in Huntington's disease: an imaging polymarker. Ann. Neurol. 83, 532–543.
Mielcarek, M., Bondulich, M.K., Inuabasi, L., Franklin, S.A., Muller, T., Bates, G.P., 2014. The Huntington's disease-related cardiomyopathy prevents a hypertrophic response in the R6/2 mouse model. PLoS One 9, 1–10.
Mihm, M.J., Amann, D.M., Schanbacher, B.L., Altschuld, R.A., Bauer, J.A., Hoyt, K.R., 2007. Cardiac dysfunction in the R6/2 mouse model of Huntington's disease. Neurobiol. Dis. 25, 297–308.
Milutinović, A., Zorc-Plesković, R., 2012. Glycogen accumulation in cardiomyocytes and cardiotoxic effects after 3NPA treatment. Bosn. J. Basic Med Sci. 12, 15–19.
Ming, L., 1995. Moldy sugarcane poisoning — a case report with a brief review. Clin. Toxicol. 33, 363–367.
Moreira, A.L., 2017. Effects of 3-Nitropropionic Acid (3-NP) on the Extrinsic Innervation of the Mice Heart- Experimental Model for Huntington's Disease. Dissertation. University of Sao Paulo.
Morton, A.J., 2018. Large-brained animal models of Huntington's disease. Methods Mol. Biol. 1780, 221–239.
Nabinger, D.D., Altenhofen, S., Bitencourt, P.E.R., Nery, L.R., Leite, C.E., Vianna, M.R.M.R., Bonan, C.D., 2018. Nickel exposure alters behavioral parameters in larval and adult zebrafish. Sci. Total Environ. 624, 1623–1633.
Palfi, S., Ferrante, R.J., Brouillet, E., Beal, M.F., Dolan, R., Guyot, M.C., Peschanski, M., Hantraye, P., 1996. Chronic 3-nitropropionic acid treatment in baboons replicates the cognitive and motor deficits of Huntington's disease. J. Neurosci. 16, 3019–3025.
Paulsen, J., Ready, R., Hamilton, J., Mega, M., Cummings, J., 2001. Neuropsychiatric aspects of Huntington's disease. J. Neurol. Neurosurg. Psychiatry 5, 310–314.
Pinho, B.R., Reis, S.D., Hartley, R.C., Murphy, M.P., Oliveira, J.M.A., 2019. Mitochondrial superoxide generation induces a Parkinsonian phenotype in zebrafish and Huntington aggregation in human cells. Free Radic. Biol. Med. 130, 318–327.
Quiñonez-Silvero, C., Hübner, K., Herzog, W., 2019. Development of the brain vasculature and the blood-brain barrier in zebrafish. Dev. Biol. 9, 0012–1606.
Rai, S.N., Singh, B.K., Rathore, A.S., Zahra, W., Keswani, C., Birla, H., Singh, S.S., Dilnashin, H., Singh, S.P., 2019. Quality control in Huntington's disease: a therapeutic target. Neurotox. Res. 11 (Review).
Ramaswamy, S., Mcbride, J.L., Kordower, J.H., 2007. Animal models of Huntington's disease. ILAR J. 48, 356–373.
Rico, E.P., Rosemberg, D.B., Langoni, A. da S., Souto, A.A., Dias, R.D., Bogo, M.R., Bonan, C.D., Souza, D.O., 2011. Chronic ethanol treatment alters purine nucleotide hydrolysis and nucleotidase gene expression pattern in zebrafish brain. Neurotoxicology 32, 871–878.
Rubinsztein, D.C., 2002. Lessons from animal models of Huntington's disease. Trends Genet. 18, 202–209.
Ryu, J.K., Nagai, A., Kim, J., Lee, M.C., McLarnon, J.G., Kim, S.U., 2003. Microglial activation and cell death induced by the mitochondrial toxin 3-nitropropionic acid: in vitro and in vivo studies. Neurobiol. Dis. 12, 121–132.
Saydoff, J.A., Liu, L.S., Garcia, R.A., Hu, Z., Li, D., von Borstel, R.W., 2003. Oral uridine pro-drug PN401 decreases neurodegeneration, behavioral impairment, weight loss and mortality in the 3-nitropropionic acid mitochondrial toxin model of Huntington's disease. Brain Res. 994, 44–54.
Schuldenzucker, V., Schubert, R., Muratori, L.M., Freisfeld, F., Rieke, L., Matheis, T., Schramke, S., Motlik, J., Kemper, N., Radespiel, U., Reilmann, R., 2017. Behavioral testing of minipigs transgenic for the Huntington gene-a three-year observational study. PLoS One 12, e0185970.
Shelbourne, P.F., Killeen, N., Hevner, R.F., Johnston, H.M., Tecott, L., Lewandoski, M., Ennis, M., Ramirez, L., Li, Z., Iannicola, C., Littman, D.R., Myers, R.M., 1999. A Huntington's disease CAG expansion at the murine Hdh locus is unstable and associated with behavioural abnormalities in mice. Hum. Mol. Genet. 8, 763–774.
Silva-Palacios, A., Ostolga-Chavarría, M., Buelna-Chontal, M., Garibay, C., Hernández-Reséndiz, S., Roldán, F.J., Flores, P.L., Luna-López, A., Königsberg, M., Zazueta, C., 2017. 3-NP-induced Huntington's-like disease impairs Nrf2 activation without loss of cardiac function in aged rats. Exp. Gerontol. 96, 89–98.
Stewart, A.M., Braubach, O., Spitsbergen, J., Gerlai, R., Kalueff, A.V., 2014. Zebrafish models for translational neuroscience research: from tank to bedside. Trends Neurosci. 37, 264–278.
Stricker-Shaver, J., Novati, A., Yu-Taeger, L., Nguyen, H.P., 2018. Genetic rodent models of Huntington disease. Adv. Exp. Med. Biol. 1049, 29–57.
Tran, S., Nowicki, M., Fulcher, N., Chatterjee, D., Gerlai, R., 2016. Interaction between handling induced stress and anxiolytic effects of ethanol in zebrafish: a behavioral and neurochemical analysis. Behav. Brain Res. 298, 278–285.
Túnez, I., Santamaría, A., 2009. Modelo de enfermedad de Huntington inducido con ácido 3-nitropropiónico. Rev. Neurol. 48, 430–434.
Túnez, I., Tasset, I., Pérez-De La Cruz, V., Santamaría, A., 2010. 3-Nitropropionic acid as a tool to study the mechanisms involved in Huntington's disease: past, present and future. Molecules. 15, 878–916.
van der Burg, J.M.M., Gardiner, S.L., Ludolph, A.C., Landwehrmeyer, G.B., Roos, A.C., Aziz, N.A., 2017. Body weight is a robust predictor of clinical progression in Huntington disease. Ann. Neurol. 82, 479–483.
Vaz, R.L., Outeiro, T.F., Ferreira, J.J., 2018. Zebrafish as an animal model for drug discovery in Parkinson's disease and other movement disorders: a systematic review. Front. Neurol. 1, 347.
Vis, J.C., De Boer-Van Huizen, R.T., Verbeek, M.M., De Waal, R.M.W., Ten Donkelaar, H.J., Kremer, B., 2004. Creatine protects against 3-nitropropionic acid-induced cell death in murine corticostriatal slice cultures. Brain Res. 1024, 16–24.
Wang, X.H., Souders, C.L., Zhao, Y.H., Martyniuk, C.J., 2018. Paraquat affects mitochondrial bioenergetics, dopamine system expression, and locomotor activity in zebrafish (Danio rerio). Chemosphere 191, 106–117.
Westerfield, M., 2000. The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (*Danio rerio*), 4th edition. University of Oregon Press, Eugene.
Wood, N.I., Morton, A.J., 2015. Social behaviour is impaired in the R6/2 mouse model of Huntington's disease. J Huntingtons Dis 4, 61–73.
Zeef, D.H., Vlamings, R., Lim, L.W., Tan, S., Janssen, M.L.F., Jahanshahi, A., Hoogland, G., Prickaerts, J., Steinbush, H.W.M., Temel, Y., 2012. Motor and non-motor behaviour in experimental Huntington's disease. Behav. Brain Res. 226, 435–439.