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Physical exercise prevents behavioral alterations in a reserpine-treated zebrafish: A putative depression model

Gabriela Madalena de Melo Martins [a,b], Barbara Dutra Petersen [a,b], Gabriel Rübensam [c], Júlia Maria Kuhl da Silva [b], Karina Vidarte Gaspary [a,b], Melissa Talita Wiprich [a,b], Stefani Altenhofen [a,b,d], Carla Denise Bonan [a,b,d,*]

[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] Centro de Pesquisa em Toxicologia e Farmacologia – INTOX, Escola de Ciências da Saúde e da Vida, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil
[d] Instituto Nacional de Ciência e Tecnologia em Doenças Cerebrais, Excitotoxicidade e Neuroproteção, Porto Alegre, RS, Brazil

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ABSTRACT

Major depressive disorder (MDD) has increasingly reached the world population with an expressive increase in recent years due to the COVID-19 pandemic. Here we used adult zebrafish (*Danio rerio*) as a model to verify the effects of reserpine on behavior and neurotransmitter levels. We observed an increase in the immobile time and time spent in the bottom zone of the tank in reserpine-exposed animals. The results demonstrated a decrease in distance traveled and velocity. Reserpine exposure did not induce changes in memory and social interaction compared to the control group. We also evaluated the influence of exposure to fluoxetine, a well-known antidepressant, on the behavior of reserpine-exposed animals. We observed a reversal of behavioral alterations caused by reserpine. To verify whether behavioral alterations in the putative depression model induced by reserpine could be prevented, the animals were subjected to physical exercise for 6 weeks. The results showed a protective effect of the physical exercise against the behavioral changes caused by reserpine in zebrafish. In addition, we observed a reduction in dopamine and serotonin levels and an increase in the 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the brain. Physical exercise was able to prevent the changes in dopamine and serotonin levels, reinforcing that the preventive effect promoted by physical exercise is related to the modulation of neurotransmitter levels. Our findings showed that reserpine was effective in the induction of a putative depression model in zebrafish and that physical exercise may be an alternative to prevent the effects induced by reserpine.

1. Introduction

Major depressive disorder (MDD) or simply depression is a disabling condition that presents a set of symptoms able to affect physical health, human relationships, and cognitive function and may lead to suicide (Brigitta, 2002; Hasler et al., 2004; DSM-5, 2013; Jesulola et al., 2018).

Several studies have hypothesized the causes of MDD for decades. The monoaminergic hypothesis, in which depression is caused by an alteration in the levels of one or more monoamines, such as serotonin (5-HT) and dopamine (DA) (Dean and Keshavan, 2017), has been the most studied. The pharmacological approach widely adopted for MDD treatment is the use of selective serotonin reuptake inhibitors (SSRI) (Bromet et al., 2011). However, studies show that the treatment interruption ranges from 15 % to 132 % depending on the drug used (Cipriani et al., 2018) and is mainly related to the occurrence of side effects, like weight gain, increased diabetes risk, sexual dysfunction, among others (Schuch and Stubbs, 2019). Studies have reported that beyond pharmacological modulation, physical exercise could also influence monoamine levels (Basso and Suzuki, 2017), becoming an alternative for depression treatment. However, the systematic review developed by Schuch et al.

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

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(2016) demonstrated there were few studies and some methodological limitations, concluding that further studies are needed to better understand the effects of physical exercise on MDD.

The zebrafish is an animal model used in studies related to psychiatric diseases, such as anxiety and depression (Nguyen et al., 2014; DePasquale and Leri, 2018; Silveira et al., 2018). This species has already identified several neurotransmitter systems related to MDD, such as dopaminergic and serotoninergic systems (Horzmann and Freeman, 2016). Behavioral symptoms of depressive-like states can also be observed, such as anhedonia (reduced reward behavior), loss or excess appetite, motor delay (hypolocomotion), fatigue, irritability, restlessness (hyperactivity), lethargy, and social and cognitive deficits (Nguyen et al., 2014). To mimic symptoms of MDD in zebrafish, some methods such as unpredictable chronic stress, social isolation, genetic or pharmacological models, as reserpine exposure-induction, can be used (Nguyen et al., 2014). Zebrafish have also been used as a model to analyze the effects of exercise on behavior and neurotransmission (Luchiari and Chacon, 2013; Gilbert et al., 2014; DePasquale and Leri, 2018; Silveira et al., 2018). Among some of the beneficial effects of physical exercise, the reduction of anxiety and improvement in the learning process have already been described in the literature on zebrafish (Luchiari and Chacon, 2013; Gilbert et al., 2014; DePasquale and Leri, 2018; Silveira et al., 2018).

Therefore, the present study aimed to establish a putative depression model induced by reserpine in adult zebrafish through neurochemical and behavioral analysis. We also tested fluoxetine to validate this model and evaluated the preventive effects of physical exercise in a putative reserpine-depression model in zebrafish.

2. Materials and methods

2.1. Animals

In this study, a total of 336 adult male zebrafish (*Danio rerio*, strain AB, 6–8 months) from our breeding colony were used. The animals were maintained in recirculating systems (Zebtec, Tecniplast, Italy) with reverse osmosis filtered 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) (Zebtec, Tecniplast, Italy). The animals were submitted to a light/dark cycle of 14/10 h respectively and received a balanced diet with commercial flakes (TetraMin Tropical Flake Fish®) three times a day that was supplemented with brine shrimp (Westerfield, 2000).

The animals were manipulated according to procedures indicated by the Brazilian Council of Animals Experimentation guidelines for Use of Fish in Research (CONCEA) and the Brazilian legislation (11.794/08). All protocols were approved by the Animal Care Committee of the Pontifical Catholic University of Rio Grande do Sul (CEUA-PUCRS, protocol number 9922). 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. Exposure to reserpine

The reserpine (RES) group was exposed to 40 μg/mL reserpine diluted in the water for 20 min (Kyzar et al., 2013; Zhang et al., 2018). The control (CTRL) group was maintained under the same conditions and exposed only to the water. After 7 days, neurochemical and behavioral analyses were performed, as shown in Fig. 1. In the following phases of the study, RES and CTRL groups were exposed to two different conditions: 1) fluoxetine treatment (FLU); or 2) physical exercise (EXER), as detailed below and shown in Figs. 2 and 3, respectively.

2.2.2. Fluoxetine treatment

For validation of the putative depression model induced by reserpine, the animals were exposed to fluoxetine at a concentration of 0.1 μg/mL for 7 days (Egan et al., 2009; Wong et al., 2013; Marcon et al., 2016; Song et al., 2018). Four groups of animals were tested: 1) CTRL group, animals exposed only to water; 2) RES group, animals exposed to reserpine; 3) FLU group, animals chronically exposed to fluoxetine; 4) FLU/RES group, animals exposed chronically to fluoxetine after reserpine treatment. FLU and FLU/RES groups were exposed for 7 days to fluoxetine dissolved in the home tank, and this solution was changed daily. The FLU/RES group was exposed to fluoxetine immediately after treatment with reserpine, as shown in Fig. 2. The RES group was exposed to reserpine and kept in the water for the subsequent 7 days for further analysis. The CTRL group remained only in water. Once the solution containing fluoxetine was changed daily, the animals of the RES and CTRL groups also had the water changed to mimic the same manipulations in all analyzed groups. After 7 days of exposure, the animals were submitted to behavioral analyses.

2.2.3. Physical exercise protocol

The exercise protocol was based on the study performed by DePasquale and Leri (2018) with modifications. The protocol consisted of 30 min of exercise a day, 3 times a week, for 6 weeks, totalizing 18 days of exercise. To analyze the effect of physical exercise in the putative reserpine-depression model, four groups of animals were tested, namely: 1) CTRL group, animals exposed only to water; 2) RES group, animals exposed to reserpine; 3) EXER group, animals submitted to physical exercise; and 4) EXER/RES group, animals exposed to physical exercise and reserpine treatment. In the EXER group, the animals were allocated in groups of 5 animals in the exercise apparatus and were submitted to the physical exercise protocol for 6 weeks; the EXER/RES group was submitted to physical exercise under the same conditions as the EXER group and it was acutely exposed to reserpine 7 days before the

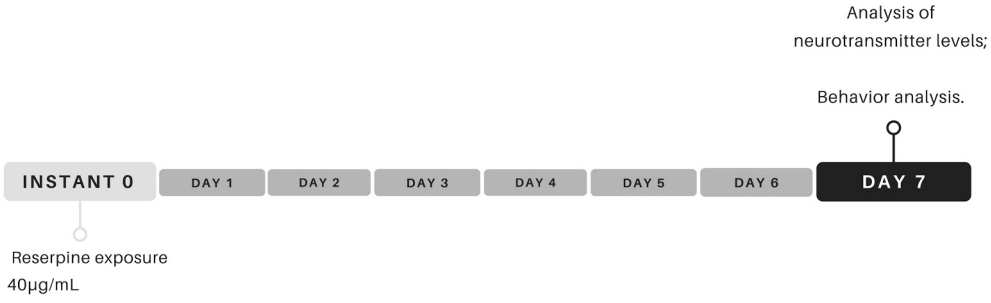

Fig. 1. Timeline of performing experimental procedures for treatment with reserpine.

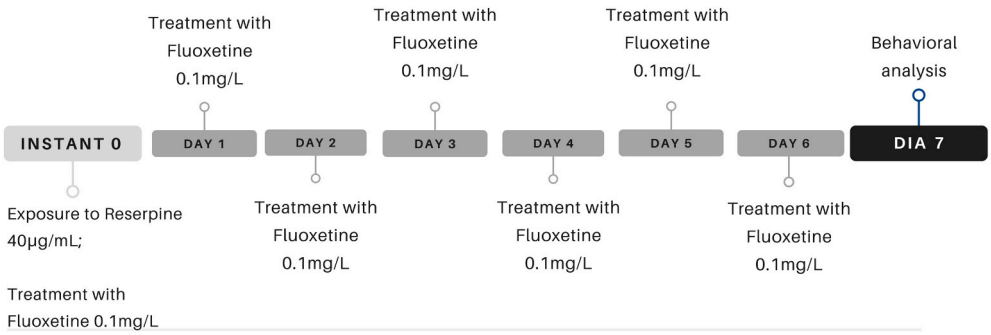

Fig. 2. Timeline of performing experimental procedures for treatment with fluoxetine in animals exposed to reserpine.

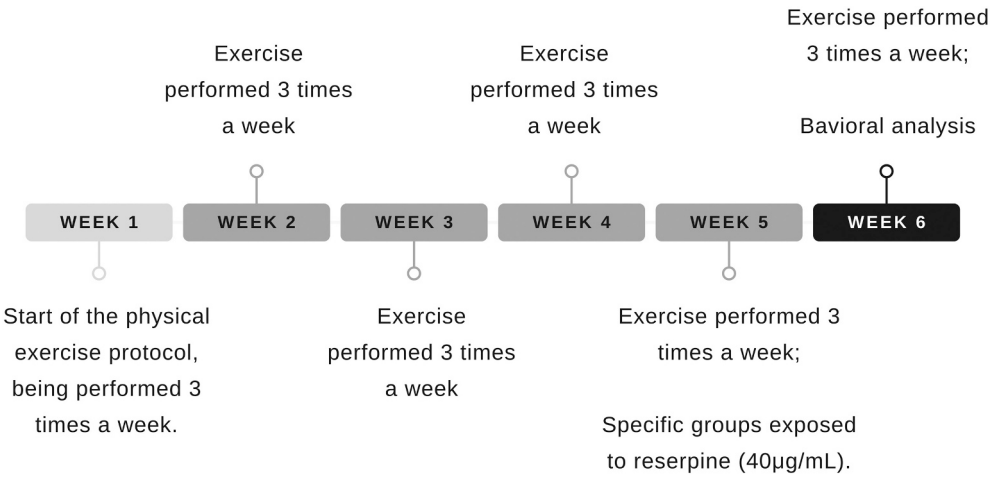

Fig. 3. Timeline of experimental procedures performed in animals submitted to physical exercise and exposed to treatment with reserpine.

behavioral tests, at a concentration of 40 µg/mL for 20 min (Fig. 3). After these 6 weeks, behavioral tests were performed.

The adult zebrafish were submitted to aerobic training by increasing the swimming speed (0.2–0.5 m/s) performed using a water pump (Ocean tech, 12,000 L/h, with flow controller). The velocity parameter was measured every day before each experiment in three parts of the flow rate (proximal, medial, and distal), using a flowmeter built by the *Laboratório de Mecânica* (*LabMec*) and *Laboratório de Computação* (*LabCOM*) of the *Centro de Apoio ao Desenvolvimento Científico e Tecnológico da PUCRS* (IDEIA). To carry out the physical exercise, a specific apparatus was used (Fig. 4). The apparatus consists of a structure composed of two tanks. The first is a reservoir containing a heater and a flow filter that controls and regulates the speed of the water. The second is the exercise tank, in which the animals were placed to perform the task. This tank has a flow standardization barrier, responsible for ensuring that the water flow speed is evenly distributed. In addition, the tank has a protective barrier to prevent animals from moving into the reservoir tank. This entire structure was fixed on aluminum support. This apparatus was based on the study by DePasquale and Leri (2018) and was made and tested by the *Laboratório de Mecânica (LabMec)* and *Laboratório de Computação (LabCOM)* of the *Centro de Apoio ao Desenvolvimento Científico e Tecnológico da PUCRS (IDEIA)*.

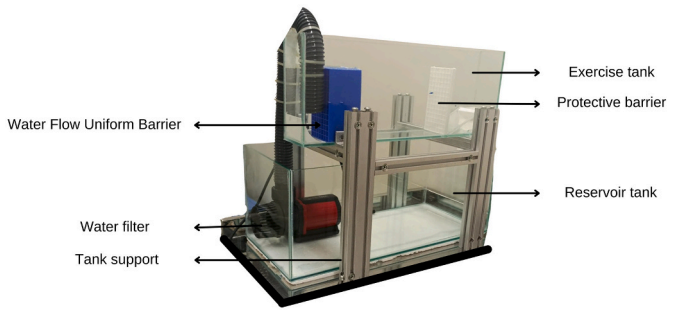

Fig. 4. Photograph of the physical exercise apparatus used in the present study (made by IDEIA-PUCRS).

2.3. Behavioral analyses

2.3.1. Novel tank test

The locomotor behavior of each animal was measured ($n = 20$). The experiments were performed in a temperature-controlled room (28 ± 2 °C) between 8:30 am and 12:00 pm. 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 immobile time (s), distance traveled (cm), velocity (cm/s, the ratio between distance traveled and movement), and time spent in the bottom zone (s). 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). The time spent in the upper zone is indicative of anxiolytic-like behavior since zebrafish tend to spend more time at the bottom zone of the tank when introduced to a new environment and then move to the upper zone after a few minutes (Levin et al., 2007).

2.3.2. Social interaction

Zebrafish are schooling fish that may exhibit a preference for their

conspecifics. Social interaction was evaluated ($n = 20$) between 8:30 am and 12:00 pm, using the same animals tested in the novel tank test. 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 60 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 two equal sections. "Social Zone" corresponded to the segments closer to the conspecific school and "Non-social Zone" was considered as the segment closer to the empty tank. The amount of time the experimental fish spent in each zone was measured during the 5 min experiment.

2.3.3. Inhibitory avoidance task

To assess if reserpine could impair memory in adult animals, we performed an inhibitory avoidance test ($n = 12$) between 9:00 am and 12:00 pm (Blank et al., 2009; Nabinger et al., 2018), with animals not previously tested in other tasks. 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 and one white. 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, 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 gently 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.

2.4. Analysis of serotonin, dopamine, and 3,4-dihydroxyphenylacetic (DOPAC) levels

For the analysis of neurotransmitter levels, liquid chromatography with a mass spectrometer (LC-MS) was used. The sample was prepared as described in other studies (Altenhofen et al., 2017; Zanandrea et al., 2020). The experiments were conducted using six samples containing a pool of six brains. Samples were separated and homogenized in 300 μL of 0.1 M formic acid (Sigma-Aldrich, St. Louis, MO) and centrifuged at 20,000 ×*g* for 20 min at 4 °C. The supernatant was transferred to a glass vial and injected into a Model 1290 Infinity UHPLC, an Agilent Model 6460 TQQQ Mass Spectrometer (all HPLC components and MassHunter software are from Agilent Technologies ®, Santa Clara, CA, USA). Chromatographic separations were performed on a Zorbax Eclipse Plus C18 RRHD, 5 × 2.1 mm, 1.8-micron column (Agilent, Paolo Alto, USA), using a mobile phase composed of (A) 0.1 % formic acid and (B) acetonitrile with 0.1 % formic acid, in gradient mode. The spectrometer was operated in MRM mode and the analytes were quantified with the following transitions, 5-HT (177 > 160), DA (154 > 119.1), and DOPAC (169 > 123 and 169 > 77). Quantifications were performed by external standardization and calibration curves were obtained with the following concentrations: DA 1.0, 5.0, 10.0, 15.0, and 20.0 ng/mL; SER 1.0, 5.0, 10.0, 15.0, and 20.0 ng/mL; GLU 0.1, 0.5, 1.0, 1.5, and 2.0 μg/mL. Standards were prepared individually, at a concentration of 0.5 mg/mL. Before the analyses, they were mixed and proposed with mobile A and added by β-mercaptoethanol in an equivalent concentration of the samples. The results were corrected for the protein concentration of the samples.

2.5. Statistical analysis

Data were expressed as mean ± standard error of the mean (S.E.M), mean ± standard deviation of the mean (S.D.), or median with the interquartile range depending on the analysis. Outliers were removed using the "Identify outliers" function of the Graphpad Prism 8.4.2 software, using the ROUT method with Q = 1 %. Data distribution was assessed for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Depending on the distribution and number of groups, one of the following statistical tests was used: Mann-Whitney, Student's *t*-test, one-way ANOVA, or the Kruskal-Wallis test. Post-hoc analyses were performed by Tukey or Dunn tests, according to the need to compare treated and control groups or between zones.

3. Results

3.1. Establishment of the MDD model

3.1.1. Behavioral and memory analysis of animals exposed to reserpine

The behavioral pattern of the animals was analyzed 7 days after a single 20- min exposure to reserpine (40 μg/mL). We observed locomotor alterations after exposure to reserpine (Fig. 5) in all parameters analyzed. Compared to the CTRL group, RES group showed an increase in the immobile time parameter ($F_{(17,17)} = 3.632$, $p < 0.0001$, Fig. 5A) and time spent in the bottom zone ($p < 0.0001$, Fig. 5B). Conversely, these animals showed a reduction in the parameters of velocity ($p < 0.0001$, Fig. 5D) and distance traveled ($F_{(19,\ 17)} = 3.500$, $p < 0.0001$, Fig. 5C).

There was no significant difference in social interaction (Fig. 5E) analyzed by the time spent in the stimulus zone ($p = 0.6830$) and memory (Fig. 5F) when comparing RES and CTRL groups.

3.1.2. Validation of the MDD model - behavioral analysis of animals exposed to reserpine and treated with fluoxetine

Since the findings showed alterations only in locomotor parameters, we performed this task after fluoxetine treatment ($n = 18$–20). The results demonstrated a reversal of the behavioral effects caused by reserpine, as shown in Fig. 6. Regarding immobile time and time spent in the bottom zone, the findings showed that fluoxetine reversed the increase in these parameters caused by reserpine ($p < 0.0001$, Fig. 6A and 6B). As a result, a reduction in the immobile time and time spent in the bottom zone on FLU ($p < 0.0001$, Fig. 6A; $p = 0.0011$, Fig. 6B) and FLU/RES ($p < 0.0001$, Fig. 6A and 6B) groups can be seen, showing similar results to the CTRL group ($p < 0.0001$; Fig. 6A and 6B). Likewise, fluoxetine treatment also altered the distance and velocity after reserpine exposure since an increase was seen in FLU ($p = 0.0253$, Fig. 6C; $p = 0.0222$, Fig. 6D) and FLU/RES ($p = 0.0094$, Fig. 6C; $p < 0.0001$, Fig. 6D) groups.

3.2. Behavioral effect of physical exercise in reserpine-MDD model

The animals were subjected to physical exercise for 18 days over 6 weeks. In the last week, the animals were acutely exposed to reserpine (40 μg/mL). Seven days after this exposure, the locomotion analysis was performed (n = 18–22) since only this task was shown to be altered after reserpine exposure. The results showed that physical exercise prevented the alterations caused by reserpine exposure, reducing symptoms compatible with stress, anxiety, and depression in zebrafish (Fig. 7). It was seen that physical exercise prevented the increase in immobile time and time spent in the bottom zone shown in RES group. The findings demonstrated a decrease in these parameters in EXER ($p = 0.0025$, Fig. 7A; $p = 0.0049$, Fig. 7B) and EXER/RES ($p < 0.0001$, Fig. 7A; $p = 0.0238$, Fig. 7B) groups, showing similar results to the CTRL group ($p = 0.0272$, Fig. 7A; $p < 0.0001$, Fig. 7B). Likewise, when analyzing the

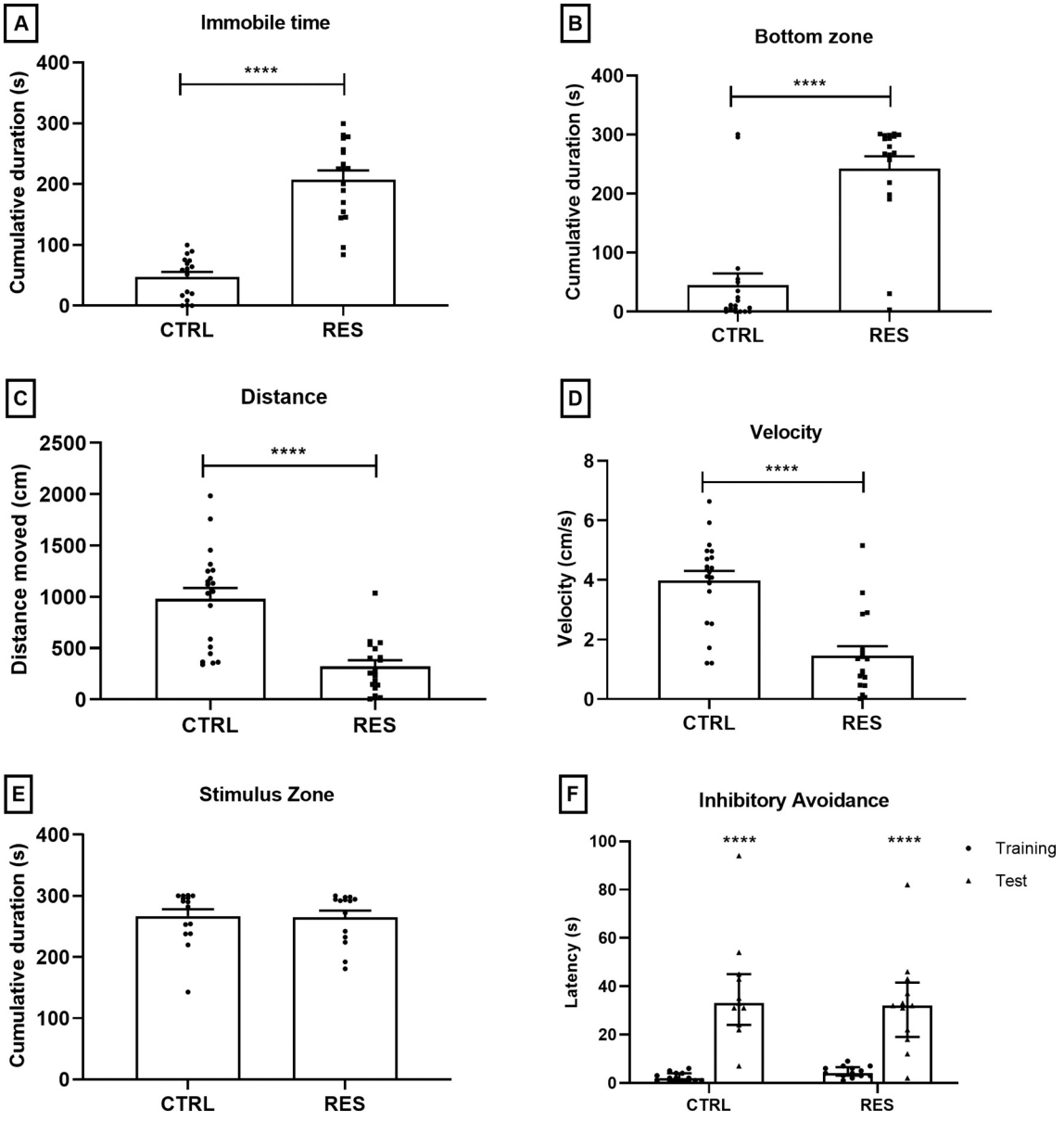

Fig. 5. Behavioral parameters of zebrafish from control and reserpine groups, after 7 days of exposure. A – immobile time (s); B – time spent in the bottom zone (s); C - distance traveled (cm); D – velocity (cm/s) ($n = 18$–20); E - social interaction measured by the time spent in the stimulus zone ($n = 18$–20); F – inhibitory avoidance memory measured by the latency between training and test sections (s) ($n = 12$). Data are expressed as mean ± S.E.M for panels A–E. For panel F, data are expressed as the median ± interquartile range. Immobile time and distance traveled were analyzed using Student's *t*-test. Velocity, time in the bottom zone, social interaction, and inhibitory avoidance task were analyzed using the Mann-Whitney test. $p \leq 0.0001$ (****) when compared with the control group.

parameters of velocity and distance, we observed that physical activity was also able to prevent the negative effects observed in the RES group. An increase in distance and velocity was observed in EXER ($p < 0.0001$, Fig. 7C and 7D) and EXER/RES groups ($p = 0.0081$, Fig. 10C; $p = 0.0002$, Fig. 7D), showing similar values to the CTRL group ($p = 0.0087$, Fig. 10C; $p = 0.0005$, Fig. 7D), while a decrease in these parameters was observed in the RES group.

3.3. Neurochemical analysis of animals exposed to reserpine

To test the effects of physical exercise on neurotransmitter levels in the reserpine-MDD model the animals were subjected to physical exercise for 18 days over 6 weeks. In the last week of physical exercise (week 6), the animals were exposed to reserpine (40 μg/mL for 20 min). Seven days after the reserpine exposure, it was performed the neurotransmitter levels analysis. We observed a significant decrease in 5-TH (RES, $F_{(1,19)} = 47.74$, $p < 0.0001$, Fig. 8A) and DA levels (RES, $F_{(1,16)} = 139.2$, $p < 0.0001$, Fig. 8B) in the RES group when compared to the CTRL group. On the other hand, an increase in the DOPAC levels in RES group was seen when compared to CTRL group (RES, $F_{(1,25)} = 51.90$, $p < 0.0001$) as shown in Fig. 8C. However, there was no significant difference in glutamate levels between the RES and CTRL groups ($F_{(1,15)} = 6.989$, $p > 0.9999$, Fig. 8D).

Interestingly, we observed a significant increase in 5-HT (RES, $F_{(1,19)} = 47.74$, $p < 0.0001$; EXER, $F_{(1,19)} = 0.5988$, $p < 0.4486$; interaction, $F_{(1,19)} = 31.31$, $p < 0.0001$, Fig. 8A), DA (RES, $F_{(1,16)} = 139.2$, $p < 0.0001$; EXER, $F_{(1,16)} = 53.59$, $p < 0.0001$; interaction, $F_{(1,16)} = 15.46$, $p = 0.0012$, Fig. 8B), and glutamate (RES, $F_{(1,15)} = 6.989$, $p = 0.0184$; EXER, $F_{(1,15)} = 7.590$, $p = 0.0147$; interaction, $F_{(1,15)} = 3.389$, $p = 0.0855$, Fig. 8D) levels in the EXER/RES group compared to RES group. On contrary, the physical exercise did not promote significant difference in DOPAC levels (RES, $F_{(1,25)} = 51.90$, $p < 0.0001$; EXER, $F_{(1,25)} =$

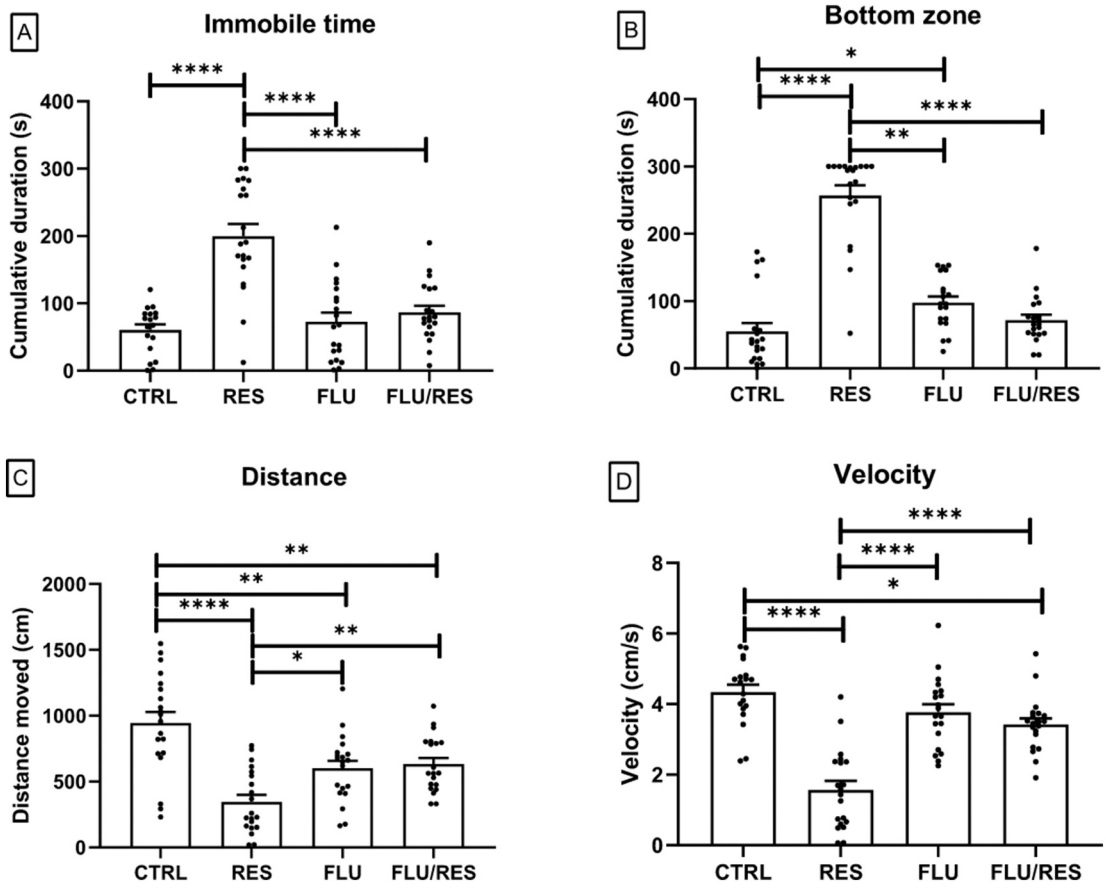

Fig. 6. Exploratory and locomotor parameters of zebrafish from the control groups, treated with fluoxetine and/or exposed to reserpine, after 7 days of exposure. A – immobile time (s); B – time spent in the bottom zone (s); C - distance traveled (cm); D – velocity (cm/s) ($n = 18$–20). Data are expressed as mean ± S.E.M. Data were analyzed by Kruskal-Wallis test, followed by post hoc Dunn test. Considered statistical difference when $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.0001$ (****).

0.3782, $p = 0.5441$; interaction, $F_{(1,25)} = 3.296$, $p = 0.0814$, Fig. 8C) in the EXER/RES group when compared to RES group (without exercise).

4. Discussion

In this study, we established a putative depression model through reserpine administration in adult zebrafish and demonstrated the effects of physical exercise on reserpine-treated zebrafish. We chose this approach since reserpine promotes monoamine depletion, which induces a rapid response when compared to other methodologies for inducing depression symptoms in this species (Kyzar et al., 2013). Also, we used the antidepressant fluoxetine to validate the putative reserpine-depression model. Afterward, we evaluated physical exercise as a potential preventive mechanism against the characteristic symptoms of depression in zebrafish, since physical exercise can cause positive alterations in the neurotransmitter levels involved in MDD (Basso and Suzuki, 2017).

Initially, we used the protocol established by Kyzar et al. (2013) for the induction of a putative depression model in zebrafish. In this protocol, we evaluated the behavior and memory of the animals after 7 days of reserpine exposure. As a result, we observed no changes in social interaction and aversive memory. However, a study conducted by Samad et al. (2021) showed that reserpine was able to cause memory deficit in rodents. This divergent result may be due to the difference in the methodology used since in this study the animals were chronically exposed to reserpine for 28 days, while in our study it was an acute exposure (20 min). We observed alterations in locomotor activity of RES groups when compared to the CTRL group. The results showed a reduction in velocity and distance traveled as well as an increase in immobile time and time spent in the bottom zone. This diminished locomotor activity, together with hypoactive exploratory behavior, indicates that reserpine mimics symptoms of depression in zebrafish. In addition, our results in the behavioral analysis are in line with other studies that have also observed alterations in the locomotion of animals that were exposed to reserpine, such as the increase in immobile time (Kyzar et al., 2013; Zhang et al., 2018; Tang et al., 2019), variations in the distance traveled and velocity (Tang et al., 2019; Zhang et al., 2018). Reserpine is a vesicular monoamine transporter (VMAT) inhibitor inducing a pathological effect due to the inhibitory action on this transporter. Tang et al. (2019) have shown a decreased expression of VMAT mRNA in the brain of zebrafish after reserpine treatment. Therefore, it is possible to suggest that the long-term behavioral effects of a single reserpine exposure observed in our study may be due to the changes in VMAT expression and irreversible blockade of VMAT promoted by reserpine, which is line with findings in zebrafish (Kyzar et al., 2013; Tang et al., 2019).

Our findings showed characteristics and symptoms presented in a depressive condition. For this reason, to validate the model in zebrafish, we used fluoxetine, an SSRI, which has its action already well characterized and is used as a treatment for depression. Our results showed that fluoxetine (0.1 μg/mL) reversed locomotor and exploratory effects caused by reserpine. These findings confirmed that behavioral symptoms found are related to a putative depression model, since fluoxetine, by itself, did not induce behavioral changes. Fluoxetine exerts antidepressant effects through an increase in synaptic serotonin. However, studies have demonstrated that fluoxetine can directly induce the phosphorylation of TrkB even in the absence of serotonin transporter (5-HTT), suggesting that this effect could be independent of 5-HT reuptake

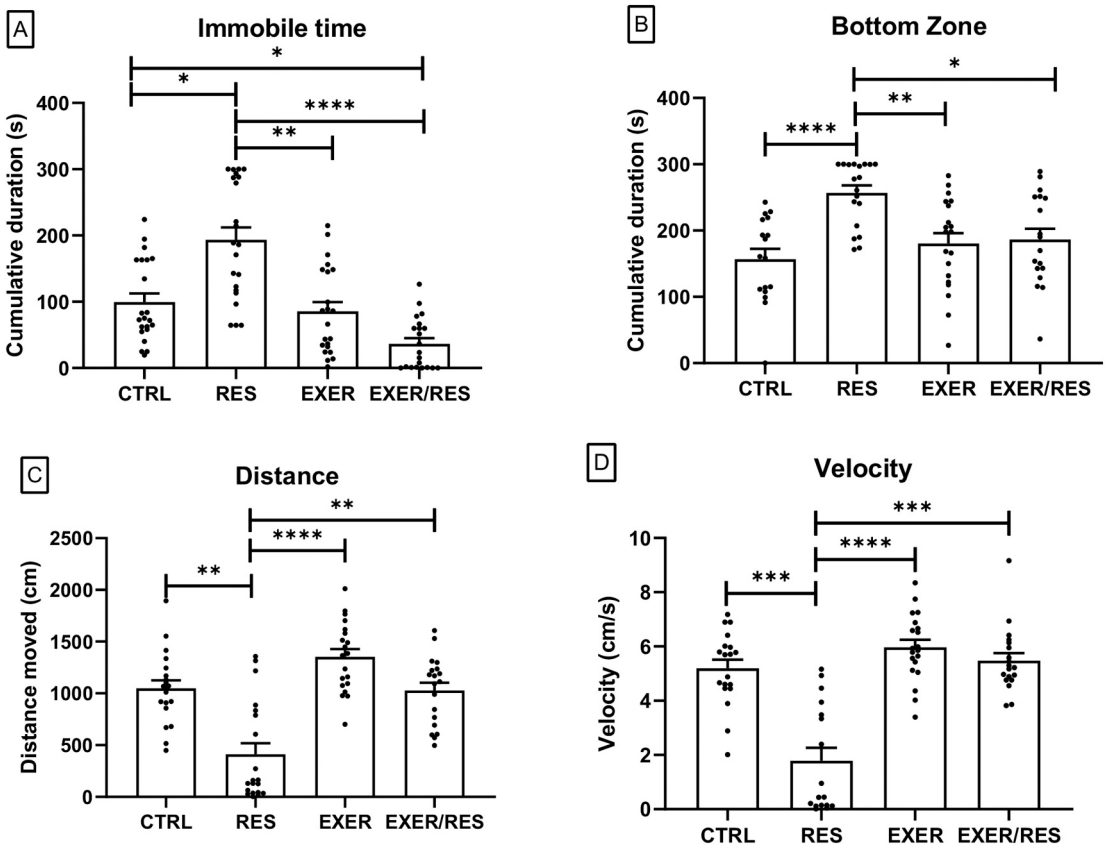

Fig. 7. Exploratory and locomotor parameters of zebrafish from the control groups, submitted to physical exercise and/or exposed to reserpine, after 7 days of exposure. A – immobile time (s); B – time in the bottom zone (s); C - distance traveled (cm); D – velocity (cm/s) ($n = 18–22$). Data are expressed as mean ± S.E.M. Data were analyzed by Kruskal-Wallis test, followed by post hoc Dunn test. Statistical difference when $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***) and $p \leq 0.0001$ (****).

blockade (Rantamäki et al., 2011). In addition, the neurogenic effects of fluoxetine have also been suggested to be partly independent of the 5-HTT blockade and have been proposed to involve other targets such as 5-HT receptors (Levy et al., 2019). Since reserpine depleted serotonin levels, it is possible that the antidepressant effects of fluoxetine observed in our study were due to serotonin-independent effects. Zhang et al. (2018) demonstrated that sertraline, an SSRI antidepressant, reversed the behavioral effects promoted by reserpine in zebrafish, which were analyzed after 7 days of treatment with sertraline in the novel tank test. These findings confirm the establishment of a putative depression model in zebrafish using reserpine as an inducer.

In addition, we evaluated how physical exercise, in a preventive way, influences the behavior of zebrafish exposed to reserpine. Previous studies have already described that physical exercise has neurochemical effects, increasing peripheral levels of DA and 5-TH in humans (Basso and Suzuki, 2017) and the brain of rodents submitted to physical exercise for 4–7 weeks (Chennaoui et al., 2000; Renoir et al., 2011; Mizutani et al., 2013). In addition, Aguiar et al. (2019) have shown that the behavioral change caused by reserpine in rodents is reversed by physical exercise performed for 20 days. These findings agree with our results showing that long-lasting physical exercise prevented the behavioral changes observed in RES group.

We also performed neurochemical analysis to assess the levels of 5-HT, DA, its metabolite DOPAC, and glutamate in reserpine-exposed zebrafish brains to check whether or not reserpine affects the neurotransmitters that are involved in MDD. The results showed a significant decrease in 5-HT and DA levels and an increase in DOPAC levels in RES group. These results were expected since reserpine acts on the vesicular monoamine transporter (VMAT) irreversibly, preventing the storage of these neurotransmitters in vesicles and causing the depletion of 5HT, noradrenaline (NE), and DA in presynaptic neurons. Monoamines are accumulated in the cytoplasm, thus there is an increase in the availability of extravesicular DA being metabolized by mitochondrial monoamine oxidase (MAO) to DOPAC, increasing the levels of this metabolite (Trejo et al., 2001). On the other hand, Tang et al. (2019) showed a decrease in 5-HT levels in zebrafish exposed to reserpine, but no changes were observed in DA levels in the same condition. However, other studies in rodents have had results similar to those observed in our study with a reduction in both 5-HT and DA levels and an increase in DOPAC levels (Roffler-Tarlov et al., 1971; Bunney et al., 1973; Oe et al., 2010). Interestingly, our findings demonstrated that physical exercise was able to prevent the changes in neurotransmitter levels since there was a significant increase in 5-HT, DA, and glutamate levels in the EXER/RES group compared to RES group. In contrast, physical exercise did not promote a significant difference in DOPAC levels in the RES group. These findings demonstrated that physical exercise was able to prevent the changes in DA, 5-HT, and glutamate levels, reinforcing that the preventive effect promoted by physical exercise on behavioral parameters is related to the modulation of neurotransmitter levels.

The zebrafish is an animal model that has gained more space for studies on exercise physiology and behavioral neurobiology. It offers many advantages over other animal models since swimming tunnels are used to rigidly control the flow speeds, which allows protocols to be standardized across studies (DePasquale and Leri, 2018). In addition, zebrafish have a natural tendency to shoal, thus facilitating the use of group exercise protocols. Another factor to be considered is that "swimming against the current" is an intrinsic factor in zebrafish, making physical exercise a non-stressful activity.

In summary, our study demonstrated that the putative depression model induced by reserpine in zebrafish showed consistent results, with

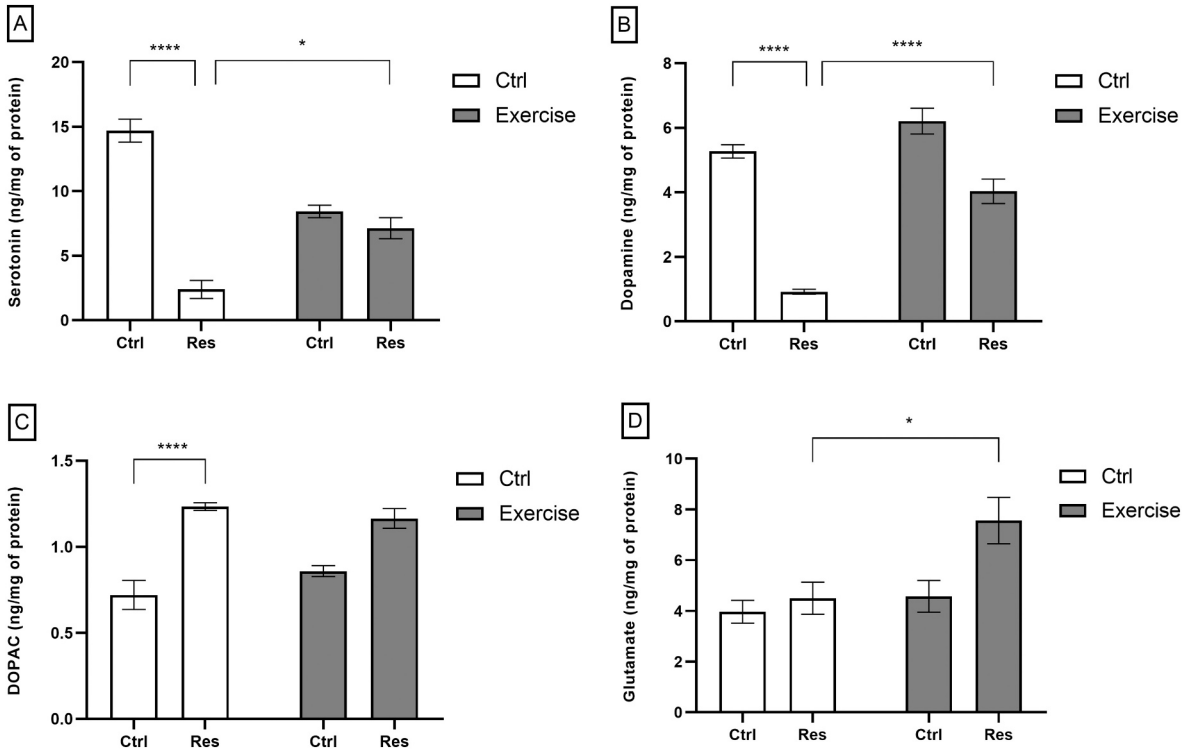

Fig. 8. Analysis of serotonin (A), dopamine (B), DOPAC (C), and glutamate (D) levels in zebrafish brains exposed to reserpine, compared with the control group (n = 4–6). Data were expressed as mean ± S.D. Data were analyzed by Student's t-test. Considered statistical difference when $p \leq 0.05$ (*), $p \leq 0.0001$ (****).

neurochemical and behavioral changes related to depression. In addition, fluoxetine and physical exercise prevented the behavioral changes induced by reserpine. These findings can open possibilities for studies of new pharmacological or physiological pathways to revert MDD using the reserpine as a model, and to explore more effects of the physical exercise in zebrafish.

Conflict of interest

The authors declare that there are no conflicts of interest.

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