



Resistance Training Modulates Hippocampal Neuroinflammation and Protects Anxiety-Depression-like Dyad Induced by an Emotional Single Prolonged Stress Model

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Abstract

Stress is a triggering factor for anxious and depressive phenotypes. Exercise is known for its action on the central nervous system. This study aimed to evaluate the role of resistance exercise in an anxiety-depression-like dyad in a model of stress. Male Swiss mice (35-day-old) were exercised, three times a week for 4 weeks on nonconsecutive days. The resistance exercise consisted of climbing a 1-m-high ladder 15 times. After mice were subjected to an emotional single prolonged stress (*Eps*) protocol. Seven days later, they were subjected to anxiety and depression predictive behavioral tests. The results showed that exercised mice gain less weight than sedentary from weeks 3 to 5. Resistance exercise was effective against an increase in immobility time in the forced swim test and tail suspension test and a decrease in grooming time of mice subjected to *Eps*. Resistance exercise protected against the decrease in the percentage of open arms time and open arm entries, and the increase in the anxiety index in *Eps* mice. Four-week resistance exercise did not elicit an antidepressant/anxiolytic phenotype in non-stressed mice. *Eps* did not alter plasma corticosterone levels but increased the hippocampal glucocorticoid receptor content in mice. Resistance exercise protected against the decrease in hippocampal levels of tropomyosin kinase B (TRKB), the p-Akt/Akt, and the p-mTOR/mTOR ratios of *Eps* mice. Resistance exercise proved to be effective in decreasing hippocampal neuroinflammation in *Eps* mice. Resistance exercise protected against the increase in the hippocampal Akt/mTOR pathway and neuroinflammation, and anxiety/depression-like dyad in *Eps* exposed mice.

Keywords Depression · Anxiety · Stress · Resistance exercise · Neuroinflammation

Introduction

Considering that the most commonly used therapy for depression is still the clinical prescription of antidepressive drugs to treat patients with major depressive disorder (MDD) and only 20 to 40% of the patients respond to these drugs [1, 2], non-pharmacological methods should be more studied, considering the safety and less-toxic side-effects. Exercise is a well-known non-pharmacological and lifestyle option

to prevent the development of many pathological states [3]. When compared to aerobic exercise modalities, resistance exercise has been less studied for its role in depression/anxiety treatment or prevention [4]. Kang et al. [5] have demonstrated that an 8-week resistance training attenuated chronic unpredictable mild stress (CUMS)-induced depression-like behaviors in male rats, suggesting that resistance exercise could be a promissory approach to even prevent or attenuate MDD and anxiety.

Approximately 350 million people worldwide experience MDD, a common neuropsychiatric condition with multiple factors involved, such as genetic and environmental, and more than 75% of people in low- and middle-income countries receive no treatment [6, 7]. The vast majority of MDD cases show high comorbidity between depression and anxiety and stress-related disorders (ASRDs), being those disorders not entirely distinct conditions in humans or animals [8, 9]. The coronavirus disease 2019 (COVID-19) elevated the

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day-by-day life stressors, including unsafety feelings, stay-at-home orders, and canceled social events [10]. According to Salari et al. [11] prevalence of stress, anxiety, and depression as a result of the pandemic in the general population is 29.6, 31.9, and 33.7% respectively.

A recent study indicates that the neurobiology of stress leads to an abnormal hypothalamic–pituitary–adrenal (HPA) axis activity [12] and neuroinflammation responses [13]. Chronic and acute stress protocols are used in rodents to induce depressive- and anxiety-like behaviors, aiming to elucidate the alterations caused by stress in central structures [14, 15].

A single prolonged stress (SPS) protocol, besides modeling a post-traumatic stress disorder, causes neuroinflammation in male mice [16]; therefore, the rationale to choose resistance exercise over other modalities of aerobic exercise is that aerobic exercise has been reported as potentially inflammatory, whereas, resistance exercise has been related to an anti-inflammatory pattern [17]. Acknowledging the potential benefits of exercise, this study evaluated if resistance exercise protects against anxiety-depression-like dyad in mice subjected to an emotional single prolonged stress (*Esp*s) protocol. The contribution of hippocampal plasticity and neuroinflammation to resistance training effects was also investigated in this model.

Materials and Methods

Animals

Male Swiss mice (aged 35 days) housed in polycarbonate cages were used in this study. They had free access to commercial feed (GUABI, RS, BRAZIL) and tap water. Animals were maintained under controlled room temperature conditions (22 ± 2 °C) and a 12-h light/12-h dark cycle, with a light cycle starting at 7:00 a.m. Animals were obtained from the Central Animal Laboratory of the Federal University of Santa Maria (UFSM)—Brazil and

handled following the rules of the Committee on Care and Use of Experimental Animals Resources of UFSM (#1,535,120,320).

Drugs

Cocktails of protease and phosphatase inhibitors and the bicinchoninic acid assay (BCA) were purchased from Sigma (Sigma-Aldrich Company, St. Louis, MO, USA). Prestained protein standard was obtained from Bio-Rad (Bio-Rad, São Paulo, Brazil). All other reagents were of analytical grade and obtained from standard commercial suppliers.

Experimental Protocol

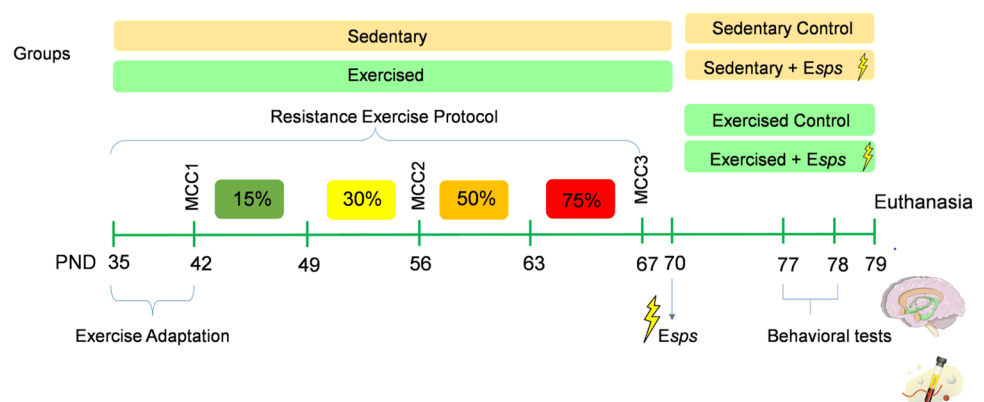
Mice were kept in the animal facility room from postnatal (PND) day 21 to 35. At PND 35, the animals were divided into two experimental groups: Sedentary (non-exercised, $n = 16$) and Exercised ($n = 16$, mice were exercised from PND 42 to 67). From the end of the resistance exercise protocol (PND 67) to the beginning of *Esp*s (PND 70), mice were not manipulated.

At PND 70, animals of both groups were divided into four groups (Fig. 1):

- Group I: Sedentary Control (non-exercised and non-stressed, $n = 8$);
- Group II: Sedentary + *Esp*s (non-exercised and subjected to *Esp*s at PND 70, $n = 8$);
- Group III: Exercise Control (exercised and non-stressed, $n = 8$);
- Group IV: Exercise + *Esp*s (exercised and subjected to *Esp*s at PND 70, $n = 8$).

After the *Esp*s protocol, mice rested until the behavioral tests (PND 77 and 78). The body weight was recorded, every 2 days, from PND 35 until PND 79.

Fig. 1 Scheme of the experimental protocol. PND postnatal day, *Esp*s emotional single prolonged stress



Resistance Exercise Protocol

Animals of groups III and IV were trained using a 1-m-high handmade ladder, following a protocol previously published [18]. At PND 35 to 42, mice were familiarized with the ladder by climbing spontaneously with no added load (exercise adaptation). The protocol was performed by mice three times a week, on Mondays, Wednesdays, and Fridays, for 4 weeks (PND 42 to 67). The mouse's body weight was measured before exercise to adjust the weight attached to the animal's tail.

For every day of training, the mice were motivated to climb the ladder 15 times with a rest of 2 min between each climb. Every week, the weight attached to the tail was progressively increased, 15% in the first week, 30% in the second week, 50% in the third week, and ending with 75% of body weight in the last week (Fig. 1). Mice were stimulated by touching their tails when they stopped climbing.

Maximum Carrying Capacity test (MCC) The maximum voluntary carrying capacity test was performed by mice based on the methodology previously adapted from rats to mice [19]. The test was carried out using a metal block mass in a small plastic microtube tied to the mouse tail. Under specific loads, mice should climb the ladder from bottom to top with less than three times of stimulation (touching their tails). We used 75% body weight as the first load of the training schedule and added 5 g sequentially if the mice could complete the task after a 5 min rest until the mice could not complete the task with three stimulations and/or even dropped down the ladder; then, the final mass value could be called the maximum capacity load. The test was performed on Friday, about 6 h after the exercise protocol, to full recovery of the animal in the adaptation week (MCC1), 30% body weight week (MCC2), and 75% body weight week (MCC3).

Emotional Single Prolonged Stress (EspS) Protocol

At PND 70, mice were individually immobilized for 2 h in an acrylic cylinder, with holes along the object to facilitate breathing. After that, each mouse was put to swim, in a cylindrical tank containing clean water at 25 °C for 15 min. To preserve animal welfare, the room was heated during the protocol, and the animals were dried with towels. They were placed in cages with excess wood shavings and remained for 15 min (recovery time) [20]. Then, the animals were subjected to the last step of the protocol, which consists of exposing the mouse to the predator odor for 3 min. The predator odor consisted of a cage with shavings, urine, and fecal pellets of male rats. The exposure was carried out in another room [21]. Animals did not have free contact with the bottom of the cage, being separated from it with a grid to avoid any contamination from rats' urine and fecal pellets. Mice

were group housed in the same cage, before and after the *EspS* protocol, to avoid exposure to an additional stressor, social isolation [22].

Non-stressed animals did not experience any stressful events. After the stress protocol animals rest for 7 days, being in touch with humans only for food and water replacement.

At PND 77 and 78, mice performed behavioral tests. At PND 79, mice were killed by cervical dislocation (Fig. 1). Hippocampus and plasma were collected and kept at -80 °C until the use. Triceps sure were also collected to determine their weight.

Behavioral tests

The behavioral tests ($n = 8/\text{group}$) were carried out in 2 days to minimize stress in the animals.

Spontaneous locomotor activity

To evaluate exploratory capacity and exclude locomotion impairment after *EspS*, mice were tested in the spontaneous locomotor test. This behavioral test was performed in a clear acrylic apparatus (50 cm × 48 cm × 50 cm) connected to a monitor with photocell beams and containing 16 infrared sensors for the automatic recording of animal position and general locomotor activity (Insight, SP, Brazil). Mice were placed in the center of the box and allowed to explore freely for 5 min. It was recorded the number of crossings, rearings, and total distance traveled.

Elevated Plus Maze (EPM)

EPM was used to evaluate anxiety-like behavior in mice, according to the method described by Pellow et al. [23]. The animals were individually placed in the central area of the maze facing an enclosed arm and observed for 5 min. The apparatus was cleaned with an ethanol solution (10% v/v) and dried with paper towels after each trial. During a 5-min test period, the number of entries in either the open or enclosed arms and the time spent in the open arms were recorded. An entry was defined as placing all four paws within the boundaries of the arm. The following measures were obtained from the test: (a) the time spent in the open/closed arms; (b) the number of entries into the open/closed arms. The percentage of time spent in the open arms, OAT% [(time spent in the open arms/total time spent in the arms) × 100], and the percentage of open arm entries, OAE% [(number of open arm entries/number of total arm entries) × 100], were calculated. The data were expressed as an anxiety index, as follows: Anxiety Index = $1 - \left[\left(\frac{\text{Open arm time}}{\text{Test duration}} \right) + \left(\frac{\text{Open arms entries}}{\text{Total number of entries}} \right) / 2 \right]$.

Tail Suspension Test

The tail suspension test (TST) was performed in a quiet experimental environment in which the total immobility duration is considered the major parameter measured to assess the “behavioral despair” rodents [24]. Mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. For the next 6 min, the latency for the first immobility episode and the immobility time was recorded. Mice were only considered immobile when passively hung and completely motionless.

Forced Swim Test

Originally described by Porsolt [25], the forced swim test (FST) is the most sensitive behavior test to evaluate the antidepressant properties of new compounds. In this test, mice were individually forced to swim in an open cylindrical container (diameter 10 cm and height 25 cm) containing 19 cm of water at 25 ± 1 °C. Latency for the first immobility episode and total immobility duration (escape-related mobility behavior) were monitored for 6 min. Each mouse was considered immobile after it ceased struggling and began floating passively on the water.

Splash Test

The splash test was adapted from [26]. This test evaluates grooming behavior, defined as cleaning of the fur by licking or scratching, after vaporization of 10% sucrose solution onto the mice’s dorsal coat. The solution’s viscosity prompts mice to initiate grooming behavior, with depressive symptoms characterized by an increased latency (idle time between spray and initiation of grooming) and decreased time spent grooming. Latency and time spent grooming were recorded for 5 min.

Biochemical Determinations

Corticosterone Assay

Blood was collected by heart puncture using heparin and samples were centrifuged at $2000 \times g$ for 10 min. Briefly, 0.2 ml of mice plasma was extracted, as well as a standard corticosterone $10 \mu\text{g/ml}$ with chloroform, centrifuged, extracted again with NaOH (0.1 M), and then exposed to fluorescence reactive without light for 2 h [27]. Fluorescence was determined and measured at 247 nm for excitation and 540 nm for emission. The fluorescence intensity was expressed as $\mu\text{g/ml}$.

Western Blot Assay

Hippocampus samples ($30 \mu\text{g}$ protein/well) and a marker protein (Bio-Rad, São Paulo, Brazil) were separated on an

SDS–polyacrylamide gel by electrophoresis. The proteins were transferred to a nitrocellulose membrane ($0.45 \mu\text{m}$, Bio-rad) using the Transfer-Blot® Turbo™ transfer system (1.0 mA, 15 to 40 min, Bio-Rad). After blocking with a 3% bovine serum albumin (BSA) solution for 1 h, the blots were incubated overnight at 4 °C with primary antibodies (Table S1). The method was performed as previously described by Müller et al. [28].

Statistical Analysis

The data were expressed as the mean \pm standard deviation (SD). Initially, data normality was verified using D’Agostino and Pearson omnibus test, excepting the proteins from the western blot, in which the Shapiro–Wilk test was used. Comparisons among experimental groups were performed by two-way analysis of variance (ANOVA) (*Esp*s and exercise) followed by Tukey’s multiple comparison test, excepting the data on the MCC test and weight, which were analyzed by repeated-measures ANOVA. Pearson’s correlation coefficient was used for measuring the linear correlation between two variables.

Results

Resistance Training Reduces the Weight Gain in Exercised Mice and Enhances the Triceps Surae Weight

The two-way analysis of body weight data demonstrated a significant difference for resistance training \times time interaction ($F_{5,150} = 16.89$; $p < 0.0001$). Exercised mice gain less weight than sedentary ones from weeks 3 to 5 (Fig. 2a) (Table S2). As shown in Fig. 2b, exercised mice increased their maximum carrying load capacity during the protocol ($F_{1,873,28.1} = 38.43$; $p < 0.0001$) (Table S2). The triceps surae weight/total body weight ratio showed no significant interaction between factors (exercise \times stress) (Table S2).

Resistance Training and Esps Do Not Induce Locomotor Alterations in Mice

Locomotor activity profile was similar among mice subjected to a resistance exercise and *Esp*s protocol (Fig. 2c, d e) (Table S3).

Resistance Exercise Protects Against Depressive-Like Phenotype in Esps Exposed Mice

Sedentary mice exposed to *Esp*s showed a statistically significant decrease in latency to immobility (Fig. 3a, $F_{1,28} = 20.02$; $p = 0.0032$) (Table S3) and an increase

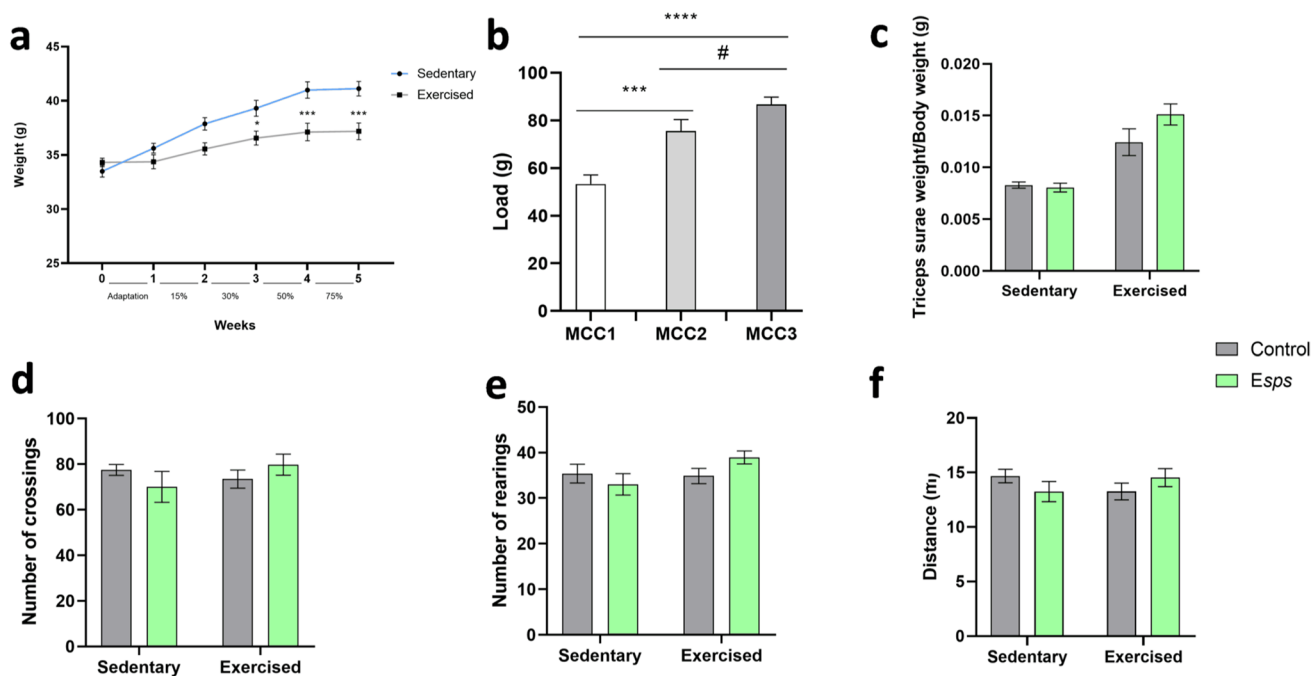


Fig. 2 Effects of resistance exercise on the body weight (**a**), maximum carrying capacity test (**b**), triceps surae weight/total body weight (**c**), the number of crossings (**d**), rearings (**e**), and distance traveled (**f**) by mice exposed to an *Esp*s protocol. Results represent the mean \pm S.E.M. of 16 mice per group (**a** and **b**) and 8 mice per group (**c–e**). * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$ compared with

a sedentary group (**a**) and compared with MCC1 (**b**), # $p < 0.05$ when compared with MCC2; two-way ANOVA with repeated measures followed by the Sidak's test (**a**); one-way ANOVA with repeated measures followed by the Tukey's test (**b**) and two-way ANOVA followed by Tukey's post hoc test (**c–e**). MCC maximum carrying capacity, *Esp*s emotion single prolonged stress

in immobility time (Fig. 3b, $F_{1,28} = 11.36$; $p < 0.0001$) (Table S3 in the TST when compared with the sedentary control group. Resistance exercise was effective against the decrease in latency to immobility ($p = 0.0149$) and the increase in immobility time ($p < 0.0001$) in *Esp*s mice.

Control exercised mice showed a decrease in latency to immobility ($p = 0.0227$) when compared to sedentary control and a decrease in immobility time ($p < 0.0001$) when compared to sedentary *Esp*s in the TST (Fig. 3a, b) (Table S3).

Exposure to *Esp*s decreased the latency to immobility (Fig. 3c, $F_{1,28} = 30.86$; $p < 0.0001$) (Table S3) in the FST in control ($p = 0.0003$) and exercise *Esp*s ($p = 0.0002$) groups when compared with a sedentary control group.

*Esp*s statistically increased the immobility time (Fig. 3d, $F_{1,28} = 9.503$; $p < 0.0001$) (Table S3) when compared with a sedentary control group in the FST, and this effect was abolished by resistance training ($p = 0.0008$).

Figure 3e shows the effects of resistance training and *Esp*s in the splash test. Sedentary mice exposed to *Esp*s had a statistically significant increase in latency to grooming ($F_{1,28} = 5.160$; $p = 0.0281$) (Table S3) and a decrease in grooming time (Fig. 3f, $F_{1,28} = 14.54$; $p = 0.0289$) (Table S3) when compared with a sedentary

control group. Resistance training protected against the increase in latency to grooming ($p = 0.0063$) and the decrease in grooming time ($p = 0.0041$) in *Esp*s-exposed mice.

Figure 3b, d, f illustrates that this resistance exercise protocol did not alter immobility time in the TST and FST, and grooming time in control mice when compared to sedentary control (Table S3).

Resistance Exercise Protects Against Anxiogenic-Like Phenotype in *Esp*s Exposed Mice

Analyses of OAT% (Fig. 4a, $F_{1,28} = 12.7$; $p = 0.0028$) (Table S3) and OAE% (Fig. 4b, $F_{1,28} = 15.14$; $p < 0.0001$) (Table S3) data revealed a significant decrease in sedentary *Esp*s exposed mice when compared with sedentary control. Resistance exercise protected against OAT% and OAE% in *Esp*s mice ($p < 0.0001$) when compared to the sedentary *Esp*s group.

Figure 4c shows that sedentary mice exposed to *Esp*s increased the anxiety index ($F_{1,28} = 13.97$; $p = 0.0029$) (Table S3) when compared with sedentary control. Resistance training protected against the increase in the anxiety index in *Esp*s ($p < 0.0001$) when compared with sedentary *Esp*s mice.

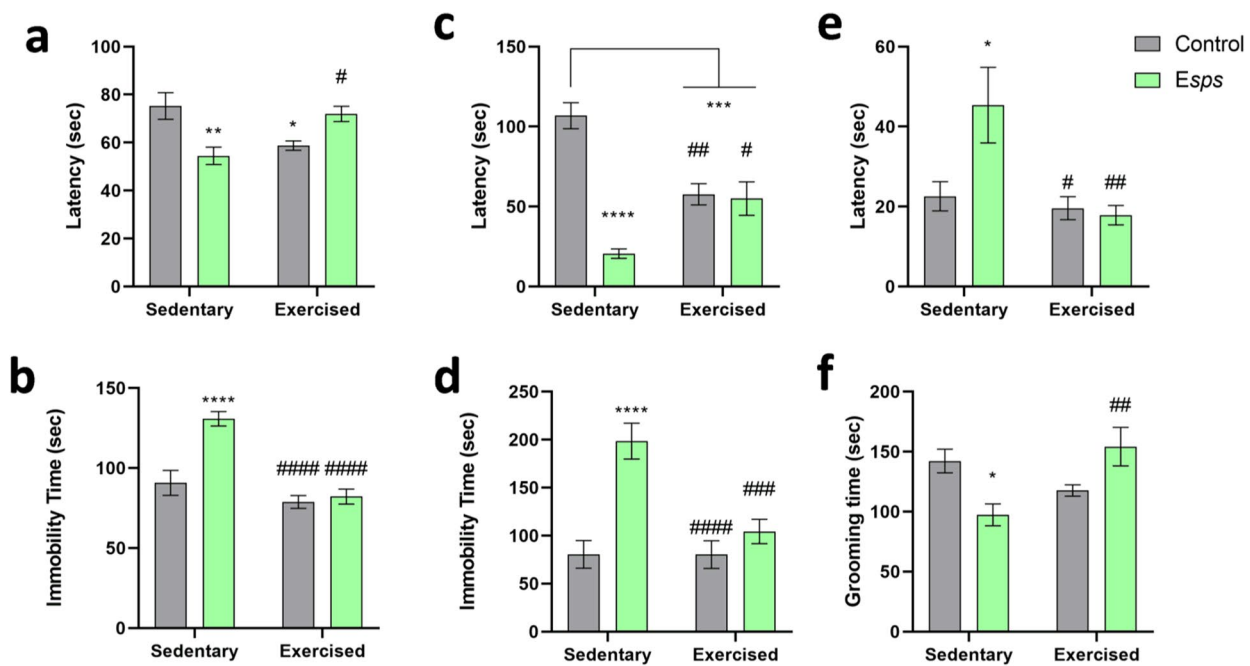


Fig. 3 Effects of resistance exercise on the TST (a, b), FST (c, d), and splash test (e, f) in mice subjected to an *EspS* protocol. Results represent the mean \pm S.E.M. of 8 mice per group. Data were analyzed by two-way ANOVA followed by Tukey's post hoc test when appro-

priate. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ compared with the sedentary control group; # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$; #### $p < 0.0001$ compared with sedentary *EspS* group. *EspS* emotion single prolonged stress

Figure 4a, b, c shows that resistance exercise protocol did not alter parameters of anxiety in control mice when compared to sedentary control (Table S3).

EspS Exposure Does Not Alter Circulating Corticosterone Levels But Increases Hippocampal GR Levels

Plasma corticosterone levels were similar in all experimental groups (Fig. 5a). The GR levels in the hippocampus of sedentary and exercised *EspS* mice were increased (Fig. 5b, $F_{1,16} = 4.573$; $p = 0.0483$) (Table S4) when compared with the sedentary control. Resistance exercise increased the levels of GR in the hippocampus of mice when compared with sedentary control.

Resistance Exercise Protects Against Hippocampal Neuroinflammation and NLRP3 Inflammasome Activation in EspS Mice

EspS increased the hippocampal levels of $\text{TNF}\alpha$ (Fig. 6a, $F_{1,16} = 4.779$; $p = 0.0377$) (Table S4), NLRP3 (Fig. 6b, $F_{1,16} = 10.26$; $p = 0.0127$) (Table S4), and IL-1 β (Fig. 6c, $F_{1,16} = 9.334$; $p = 0.0114$) (Table S4) in sedentary mice when compared with sedentary control. Resistance exercise protected against the increase of $\text{TNF}\alpha$ ($p = 0.0114$), NLRP3 ($p = 0.0019$), and IL-1 β ($p = 0.0063$) levels in the

hippocampus of mice exposed to *EspS* when compared with the sedentary *EspS* group. Resistance exercise protocol did not alter hippocampal levels of $\text{TNF}\alpha$, NLRP3, and IL-1 β in control mice (Fig. 6a-c) when compared to sedentary control.

Resistance Exercise Protects Against the Decrease in Hippocampal TRkB Signaling in EspS Exposed Mice

Data showed that sedentary mice's exposure to *EspS* led to a decrease in hippocampal TRkB levels (Fig. 7a, $F_{1,16} = 5.504$; $p = 0.0471$) (Table S4) when compared to a sedentary control group. Resistance exercise protected against the decrease in levels of TRkB in the hippocampus of mice exposed to *EspS* ($p = 0.0005$) when compared with sedentary *EspS*. Resistance exercise did not alter hippocampal TRkB levels in control mice when compared to sedentary control (Fig. 7a). proBDNF/mature BDNF ratio was similar in the hippocampus of mice from all experimental groups (Fig. 7b) (Table S4).

Figure 8a shows that the p-Akt/Akt ratio was decreased by *EspS* exposure in the hippocampus of sedentary mice ($F_{1,16} = 22.54$; $p < 0.0001$) (Table S4) when compared to the control sedentary group. Resistance exercise was effective against the decrease in the p-Akt/Akt ratio when compared to the sedentary *EspS* group ($p = 0.0362$).

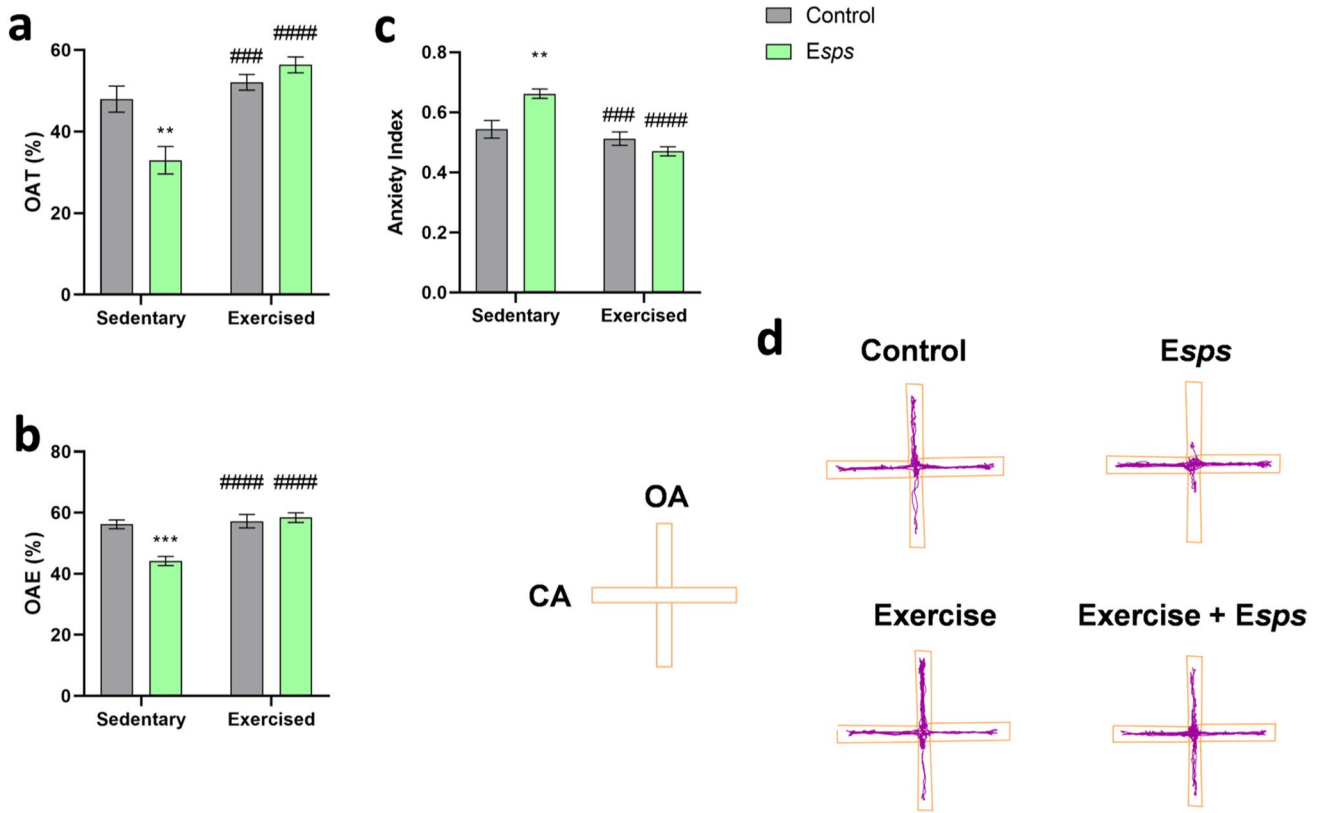
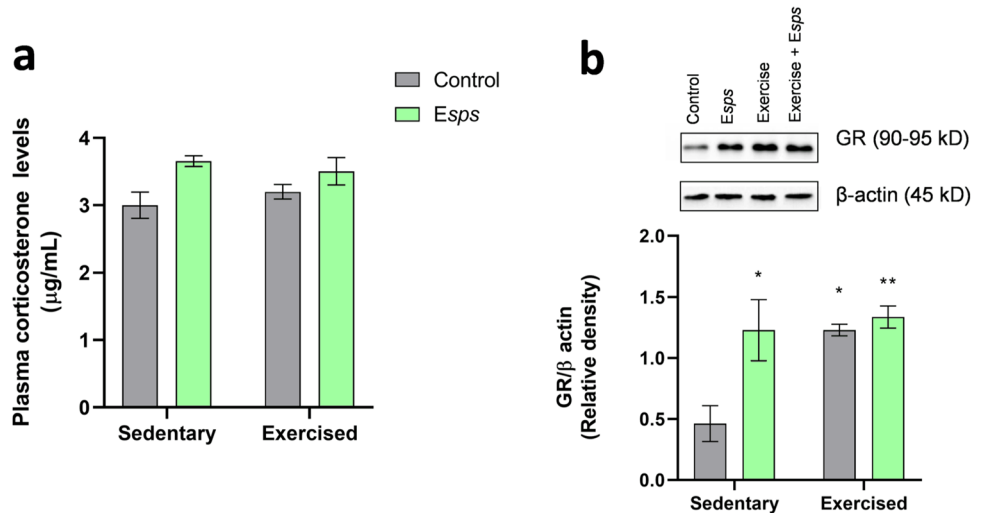


Fig. 4 Effects of resistance exercise on OAT% (a), OAE% (b), and anxiety index (c) in mice subjected to an *Esp*s protocol. Representative images (d) of animal performance in the EPM. Results represent the mean \pm S.E.M. of 8 mice per group. Data were analyzed by two-way ANOVA followed by Tukey's post hoc test.

** $p < 0.01$; *** $p < 0.001$; compared with the sedentary control group; ### $p < 0.001$; #### $p < 0.0001$ compared with the sedentary *Esp*s group. *Esp*s emotion single prolonged stress, OAT% open arms time, OAE% open arm entries, CA closed arms, OA open arms

Fig. 5 Effects of resistance exercise on plasma corticosterone levels (a) and GR levels in the hippocampus (b) of mice exposed to an *Esp*s protocol. Results represent the mean \pm S.E.M. of 8 (a) or 5 (b) mice per group. Data were analyzed by two-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$; ** $p < 0.01$ compared to the sedentary control group. *Esp*s emotion single prolonged stress, GR glucocorticoid receptor



The ratio of p-mTOR/mTOR was reduced in the hippocampus of *Esp*s ($F_{1,16} = 12.83$; $p = 0.0427$) (Table S4) mice when compared with a sedentary control group. Resistance exercise increased the hippocampal p-mTOR/mTOR ratio in *Esp*s exposed mice ($p < 0.0001$) when

compared with the sedentary *Esp*s group ($p < 0.0001$) (Fig. 8b).

Resistance exercise did not modify hippocampal p-Akt/Akt and p-mTOR/mTOR ratios in control mice when compared to sedentary control (Fig. 8a,b).

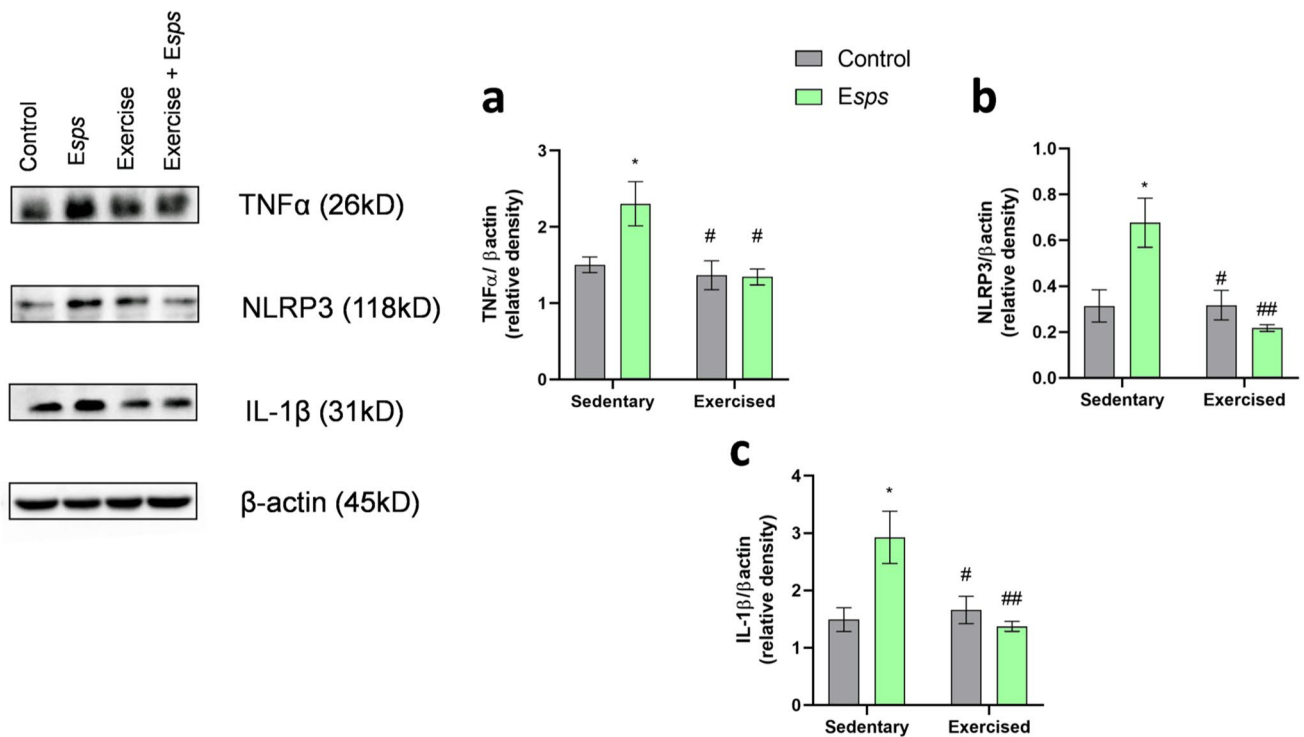


Fig. 6 Effects of resistance exercise on the levels of TNF α (a), NLRP3 (b), and IL-1 β (c) in the hippocampus of mice exposed to an Esps protocol. Results represent the mean \pm S.E.M. of 5 mice per group. Data were analyzed by two-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$ when compared to the sedentary control

group; # $p < 0.05$; ## $p < 0.01$ when compared to the sedentary Esps group. Esps emotion single prolonged stress, TNF α tumor necrosis factor-alpha, NLRP3 NOD-, LRR- and pyrin domain-containing protein 3, IL-1 β interleukin 1-beta

Fig. 7 Effects of resistance exercise on TRkB levels (a) and the BDNF ratio (b) in the hippocampus of mice exposed to an Esps protocol. Results represent the mean \pm S.E.M. of 5 mice per group. Data were analyzed by two-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$ when compared to the sedentary control group; ## $p < 0.01$; ### $p < 0.001$ when compared to the sedentary Esps group. Esps emotion single prolonged stress, TRkB tropomyosin-related kinase receptor, BDNF brain-derived neurotrophic factor

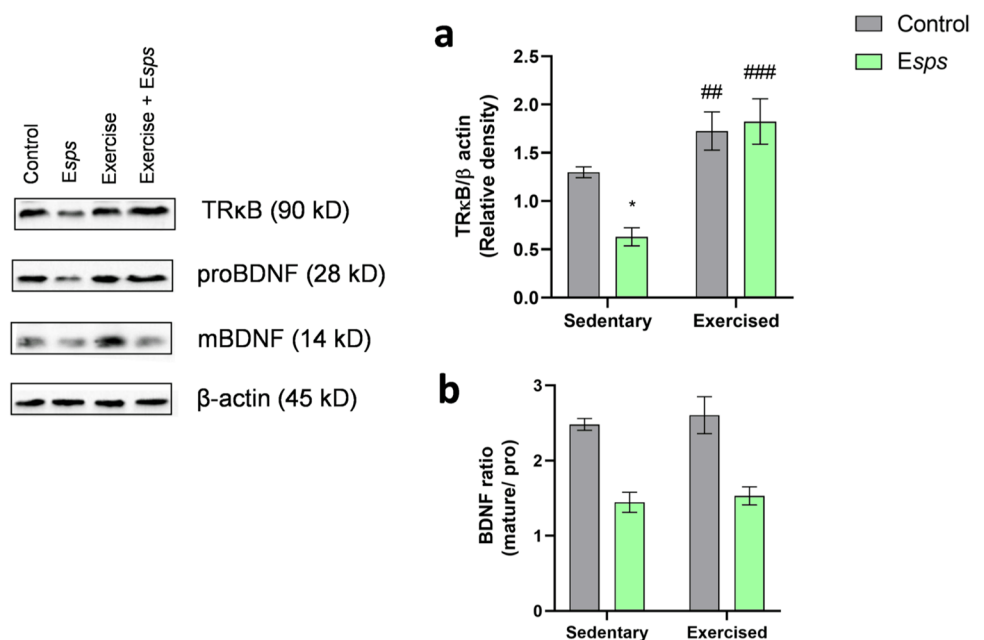
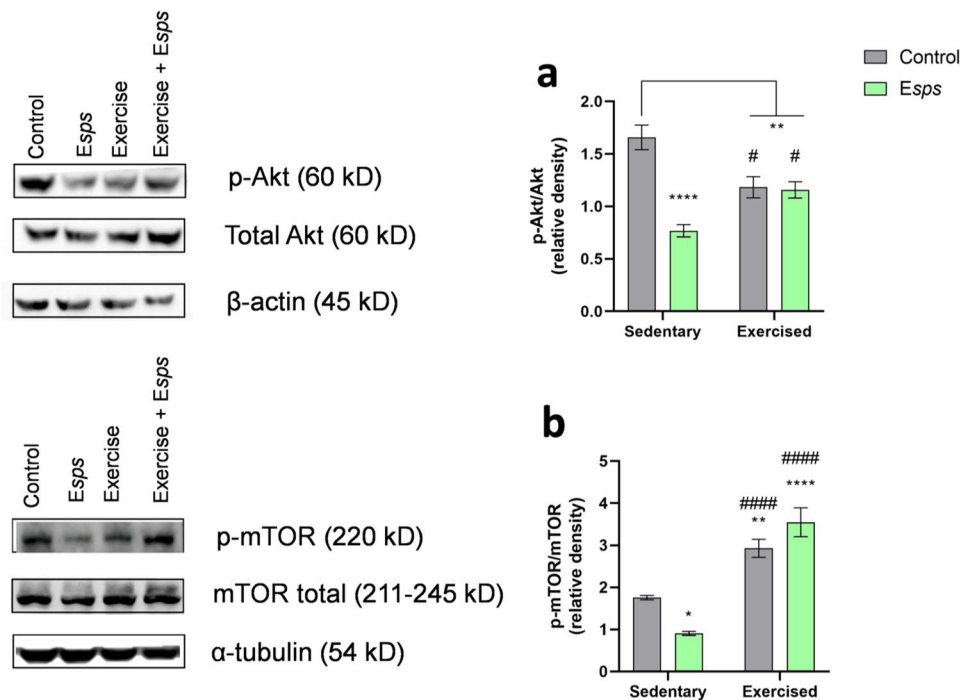


Fig. 8 Effects of resistance exercise on Akt (a) and mTOR (b) protein contents in the hippocampus of mice exposed to an *Esp*s protocol. Results represent the mean \pm S.E.M. of 5 mice per group. Data were analyzed by two-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$ when compared to the sedentary control group; # $p < 0.05$; ### $p < 0.0001$ when compared to sedentary *Esp*s group. *Esp*s emotion single prolonged stress, Akt protein kinase B, mTOR mammalian target of rapamycin



Anxiety/Depressive Phenotype Positively Correlates with Hippocampal Neuroinflammatory Markers

Pearson's analyses demonstrated statistically significant positive correlations between the anxiety index and hippocampal levels of NLRP3 ($r = 0.7716$, $p < 0.0001$), IL-1 β ($r = 0.5118$, $p = 0.0211$), and TNF- α ($r = 0.4931$, $p = 0.0272$).

Statistically significant positive correlations were found between immobility time in the FST and hippocampal levels of TNF- α ($r = 0.6429$, $p = 0.0022$), NLRP3 ($r = 0.5239$, $p = 0.0177$), and IL-1 β ($r = 0.5446$, $p = 0.0130$). Regarding the analyses between immobility time in the TST and hippocampal levels of inflammatory markers, significant positive correlations were also found (TNF- α ($r = 0.6036$, $p = 0.0048$), NLRP3 ($r = 0.5566$, $p = 0.0108$), and IL-1 β ($r = 0.4875$, $p = 0.0293$)).

Discussion

Our findings indicate an anxiety/depression-like phenotype in sedentary mice subjected to the *Esp*s protocol, which was accompanied by a hippocampal increase of inflammatory cytokine levels, activation of the NLRP3 inflammasome, a decrease in TR κ B levels, and downregulation of the Akt/mTOR pathway. Resistance exercise protected against the development of anxiety/depression-like dyad, neuroinflammation, activation of the inflammasome, and modulated the TR κ B levels and Akt/mTOR pathway in the hippocampus of *Esp*s exposed mice (Fig. 9).

Despair- and anxiety-like behaviors were observed after 7 days of *Esp*s exposure, which reflect consistency with the other stress model outcomes [29, 30]. The increase of GR protein content in the hippocampal region of rats has been reported after exposure to a modified SPS protocol [31, 32]; our results on GR hippocampal levels are also consistent with this feature in mice exposed to *Esp*s. Neuroinflammation is commonly related to depression-like behaviors in animals. This way, our data demonstrate an increase in NLRP3, TNF α , and IL-1 β levels in the hippocampus of anxious/depressed mice exposed to *Esp*s, suggesting a microglial superactivation and the release of pro-inflammatory cytokines. Accordingly, Zhang et al. [33] reported that the NLRP3 inflammasome plays a critical role in stress-induced depression. Brain-derived neurotrophic factor (BDNF) exerts its signaling through activating tropomyosin receptor kinase B (TR κ B), which activates proteins involved in cell survival and migration pathways [34]. In agreement with a previously published study [32] that shows a decrease in the hippocampal TR κ B/BDNF signaling of rodents exposed to the SPS protocol, the present results reveal a decrease in the protein content of TR κ B and the BDNF ratio in the hippocampus of anxious/depressive-like mice subjected to *Esp*s.

The BDNF-TR κ B interaction activates intracellular signaling cascades, among them the Akt/mTOR pathway. Once phosphorylated, Akt activates mTOR phosphorylation, which will control the expression of proteins correlated with neuronal proliferation or survival [35]. Our findings demonstrate that hippocampal protein content of the Akt/mTOR

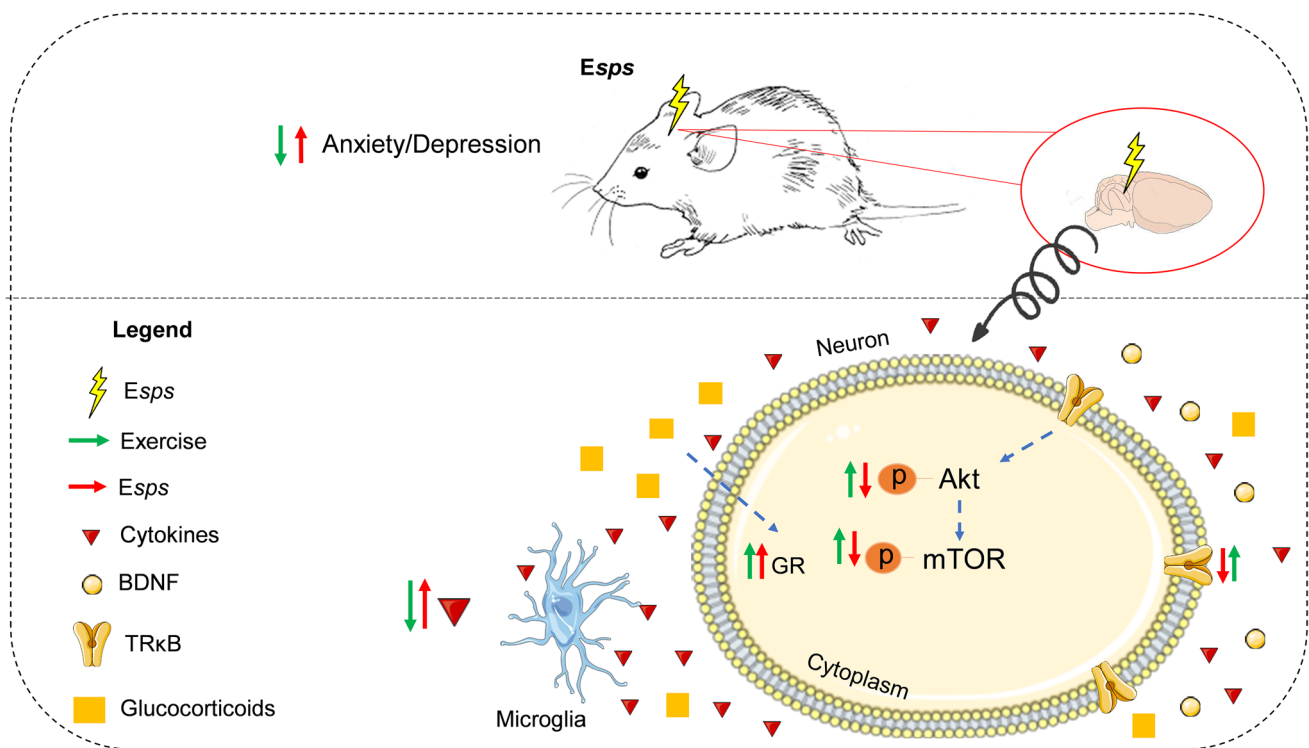


Fig. 9 Summary of *Esp*s (red arrows) and resistance exercise (green arrows) effects on neuroinflammation and Akt/mTOR pathway and its signaling in the hippocampus of mice. *Esp*s emotion single prolonged

stress, GR glucocorticoid receptor, TRkB tropomyosin-related kinase receptor, BDNF brain-derived neurotrophic factor, Akt protein kinase B, mTOR mammalian target of rapamycin

phosphorylation ratio was decreased in anxiety/depressive-like mice exposed to *Esp*s. Accordingly, a decrease in ratios of p-Akt/Akt and p-mTOR/m-TOR has been reported in the hippocampus and cerebral cortex of mice, and the silencing of this pathway has been directly related to depression-anxiety-like behaviors in rodents [36, 37].

Resistance exercise is less explored if compared with aerobic modalities as a non-pharmacological tool to protect against depressive and anxiety phenotypes. Our present data reveal that resistance training performed by mice for 4 weeks protected against anxiety/depression-like dyad in mice subjected to *Esp*s. The increase of hippocampal GR levels has been reported in young male mice subjected to an exercise protocol, which is in agreement with our present results; however, it is not clear why GR expression was also increased in *Esp*s (sedentary and exercised). It is possible that exercised mice have HPA-axis changes faster under stress [38]. Evidence has been found to suggest that exercise suppresses NLRP3 inflammasome action and modulates neuroinflammation [39]. Mice subjected, in this study, to 4 weeks of resistance exercise showed lower levels of hippocampal NLRP3. Resistance exercise decreased inflammatory markers in the cerebral cortex and hippocampus of rats exposed to a chronic stress protocol when compared to the ones in an aerobic protocol [40].

Evidence indicates that aerobic exercise increases the hippocampal levels of BDNF in rodents, whereas, resistance exercise for 8 weeks did not change this parameter, acting through the insulin-like growth factor 1 (IGF-1)/Akt pathway [41, 41]. On the other hand, TRkB modulation in the hippocampus and cerebral cortex was associated with an anxiolytic-like behavior of mice in the EPM [42]. Exercise enhances TRkB levels in the mouse hippocampus [43, 44], which can explain the increase in the levels of this protein in exercised groups. Some studies reported the importance of TRkB/m-TOR signaling as a target to potential antidepressant molecules, given that this pathway contributes to protein synthesis required for synaptic plasticity [45, 46]. Our data reveal that resistance exercise for 4 weeks modulated the hippocampal Akt-m-TOR pathway in mice exposed to *Esp*s. In agreement with our results, a comparison between aerobic and resistance exercise modalities has shown that 8 weeks of exercise lessen depression behavior and reduced hippocampal neuronal apoptosis by acting differently, aerobic exercise activates the PGC1 α /ERR α /FNDC5 pathway, whereas, resistance exercise up-regulates the IGF-1/Akt/mTOR signaling pathway in a CUMS-induced depression model in rats [5]. Although in our present study the IGF-1 has not been measured, the increase in the hippocampal GR levels could be associated

with IGF-1, a mediator of vulnerability or resilience to stress in rats [47].

An intriguing result of this study was the similarity in plasma corticosterone levels of mice from *Esp*s (sedentary and exercised) groups. Tentatively, the method used to measure corticosterone levels, which is based on the lowest sensitivity fluorimetric detection, instead of enzyme-linked immunosorbent assay and a radioimmunoassay, could be a way to explain this result.

Regarding the influence of developmental factors on stress and exercise effects in young male mice, the experimental design of this study was based on other studies using SPS, which were carried out predominantly with male mice aged from 8 to 12 weeks [48]. In the present protocol, 10-week-old mice were subjected to the *Esp*s protocol. Considering that puberty in male mice occurs around PND 40 [49], this period coincides with the HPA axis maturation and its development, suggesting that rodents can be a valid model to investigate the outcomes of stress [50]. Although there is little information on how exercise is influenced by the developmental period in rodents, the beneficial effects of early life exercise on hippocampal synaptic plasticity, excitability, and memory have been reported [51].

Of particular importance is the experimental protocol validity because of the possibility to translate preclinical results to clinical settings. Stressful episodes in early life may act as a risk factor for the development and maintenance of psychiatric disorders, such as posttraumatic stress syndrome, anxiety, and depression [52]. Resistance exercise rises as a more psychologically rewarding modality when compared with other exercise modalities, besides its results in improving psychological aspects of health, such as depression, self-esteem, anxiety, and stress [53]. In general lines, this study demonstrates the impact of resistance exercise on anxiety/depression dyad and hippocampal neuroinflammatory markers in young male mice subjected to the *Esp*s protocol, an experimental model that recapitulates in rodents some of the core symptoms of post-traumatic stress disorder. Moreover, the behavioral outcomes were positively correlated with molecular results reinforcing our hypothesis.

Results of behavioral tests can be affected by many factors, such as poorly standardized experimental protocols, environmental and biological factors, and human interference [54]. In this study, the variability of outcomes between behavioral tests was minimal. Among the efforts to decrease the variability, we highlight the behavioral tests conducted by researchers trained and unaware of the experimental groups.

Regarding the per se effects of resistance exercise, the present protocol reveals that it did not elicit an anxiolytic/antidepressant-like phenotype but increased the hippocampal GR levels in mice. The response of glucocorticoids to exercise is a well-known phenomenon, being the increase

in corticosterone and GR levels following resistance training something acceptable [55, 56]. We emphasize that the lack of anxiolytic/antidepressant-like effects of resistance exercise in this protocol was not attributed to detraining, considering that, short-term detraining is not considered to interfere in pathways linked to mTOR and protein kinase phosphorylation [57].

Conclusion

In the present protocol, resistance exercise was a prophylactic tool to protect against the onset of anxiety/depression-like dyad in mice exposed to *Esp*s associated with the decrease of hippocampal levels of inflammatory cytokines, NLRP3 inflammasome, and modulation of Akt/mTOR pathway. More studies are necessary to elucidate the mechanisms behind resistance exercise to abolish anxiety/depressive-like phenotype in an *Esp*s model.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12035-022-03069-x>.

Author Contribution JTKJ and GZ conceived and designed the study. JTKJ, LSM, GL, and VAZ carried out the experiments and collected the data. Data were analyzed by JTKJ, LSM, GZ, and CWN. JTKJ, GZ, and CWN revised the graphs. JTKJ and CWN wrote the first draft of the manuscript. All authors commented on the previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Ethics Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study was approved by the Committee on the Ethics of Animal Experiments of the Federal University of Santa Maria, Brazil (Permit Number: 1535120320) and carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All efforts were made to reduce the number and suffering of animals to a minimum.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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