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Pharmacology, Biochemistry and Behavior 198 (2020) 173018

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

The effect of voluntary wheel running on the antioxidant status is dependent on sociability conditions

Mauricio P. Cunha[a,*], Daniele G. Machado[a,b], Gianni Mancini[a], Viviane Glaser[a,c], Roberta de Paula Martins[a], Andreza F. de Bem[a], Alexandra Latini[a], Alcir L. Dafre[a], Ana Lúcia S. Rodrigues[a]

[a] Department of Biochemistry, Federal University of Santa Catarina, 88040-900 Florianópolis, SC, Brazil
[b] Institute of Basic Health Sciences, Department of Biochemistry, Federal University of Rio Grande do Sul, 900035-003 Porto Alegre, RS, Brazil
[c] Department of Biological and Agronomic Sciences, Federal University of Santa Catarina, 89520-000 Curitibanos, SC, Brazil

ARTICLE INFO

Keywords:
Glutathione
Mitochondria
Oxidative stress
Physical activity
Voluntary wheel running
Social isolation stress

ABSTRACT

Voluntary wheel running is widely used as a physical activity (PA) model in rodents, but most studies investigate the beneficial effects of this intervention in socially isolated mice. Social isolation stress (SIS) is associated with vulnerability to oxidative stress and reduced mitochondrial activity. Thus, the aim of this study was to investigate the effects of free access to a running wheel for 21 days on the various markers of the cellular redox/antioxidant status as well as mitochondrial function of mice subjected to SIS or maintained in groups of 3 in the homecage. SIS increased thiobarbituric acid reactive substance (TBARS) levels in the cerebral cortex, and PA intervention was not able to reverse such alteration. PA reduced TBARS levels in the liver of grouped mice and gastrocnemius of socially isolated mice. PA increased nonprotein thiol (NPSH) levels in the cerebral cortex of grouped mice. Furthermore, socially isolated mice presented lower glutathione peroxidase (GPx) activity in the cerebellum and gastrocnemius, and glutathione reductase (GR) activity in the cerebral cortex and liver. By contrast, SIS induced higher GPx activity in the cerebral cortex and heart. PA reduced GPx (cerebral cortex) and GR (cerebral cortex and liver) activities of socially isolated mice. SIS caused higher activity of mitochondrial complexes I and II in the cerebral cortex, and the PA paradigm was not able to alter this effect. Interestingly, the PA produced antidepressant-like effect at both SIS and control groups. In conclusion, the results showed the influence of SIS for the effects of PA on the antioxidant status, but not on the mitochondrial function and emotionality.

1. Introduction

Physical inactivity, also known as sedentarism, is a primary cause of most chronic diseases (Booth et al., 2012; Garber et al., 2011). Conversely, physical activity (PA) produces a number of beneficial health effects, including a reduction in the risk of several diseases related to oxidative stress and mitochondrial dysfunction, such as cancer, osteoporosis, obesity, cardiovascular disabilities, dementias, depression, and anxiety, among others (Booth et al., 2012; Kessels et al., 2018; Langsetmo et al., 2012; Rosa Jr et al., 2017; Schuch et al., 2016; Stoner et al., 2016; Stonerock et al., 2015; Thuné-Boyle et al., 2012; Zhang et al., 2018).

Running wheel exposure is commonly employed as a voluntary PA model in rodents, since this protocol is uncomplicated, easy, and results in a quantifiable measure of PA (Sherwin, 1998). Although there is no consensus on the ground mechanisms controlling running wheel activity (Novak et al., 2012), use of a running wheel increases the average rodent life expectancy by nearly 10% (Holloszy, 1998); produces antidepressant- and anxiolytic-like effects (Cunha et al., 2013; Mazur et al., 2017), which are paralleled to adult neurogenesis and neuronal survival

Abbreviations: DTNB, 5,5-dithiobis-2-nitrobenzoic acid; ANOVA, Analysis of variance; CHP, Cumene peroxide; Ethylenediamine tetraacetic acid (EDTA), Ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (also known as Egtazic acid, EGTA); GPx, Glutathione peroxidase; GR, Glutathione reductase; MDA, Malondialdehyde; NADH, Nicotinamide adenine dinucleotide; NADPH, Nicotinamide adenine dinucleotide phosphate; NPSH, Nonprotein thiols groups; GSSG, Oxidized glutathione; PA, Physical activity; GSH, Reduced glutathione; SIS, Social isolation stress; TST, Tail suspension test; TBARS, Thiobarbituric acid reactive species

* Corresponding author.
E-mail address: mauricio.personal@gmail.com (M.P. Cunha).

https://doi.org/10.1016/j.pbb.2020.173018
Received 11 February 2020; Received in revised form 14 August 2020; Accepted 17 August 2020
Available online 20 August 2020

(van Praag et al., 1999a, 1999b); cardioprotection (Bronikowski et al., 2003; Naderi et al., 2015); hepatoprotection (Bay et al., 2017; Zolfagharzadeh and Roshan, 2013); and skeletal muscle trophism (Brooks et al., 2018; Takigawa et al., 2019). Studies have indicated that running wheel exposition exerts its positive effects on health through a variety of biological pathways, but the exact mechanisms underlying its influences on health are not fully determined. In this regard, evidence suggests that the running wheel improves mitochondrial function, enhances the antioxidant system, and reduces oxidative stress in experimental models of pathologies (Aguiar et al., 2014; Cunha et al., 2013; Dantas de Lucas et al., 2014; Naderi et al., 2015; Navarro et al., 2004; Wright et al., 2007).

Most studies analyzing the health impact of running wheels employed socially isolated animals during the entire experimental protocol, or at least over certain periods of time (e.g., during training). This procedure allows the monitoring of individual variables associated to PA, such as distance travelled, time spent on the running wheel, and speed. The literature data extensively reports that social isolation stress (SIS) or lack of social support may be associated with negative health outcomes, increasing the risks for mortality (Broadhead et al., 1983; House et al., 1988). In accordance with this, preclinical studies indicated that SIS induces behavioral abnormalities such as anxiety (Butler et al., 2014; Skelly et al., 2015) and depression (Zanier-Gomes et al., 2015), cognitive dysfunction (Li et al., 2016), cardiomyopathy (Sonei et al., 2017), impairment in the response to stroke and ischemia (Karelina et al., 2009a, 2009b; O'Keefe et al., 2014; Venna et al., 2014), obesity and metabolic disturbance (Koshoridze et al., 2016; Sun et al., 2014), oxidative stress (reviewed by Filipović et al., 2017; Mumtaz et al., 2018; Shao et al., 2015; Zlatković et al., 2014a, 2014b), and mitochondrial dysfunction (Zhuravliova et al., 2009). Additionally, lack of social support is etiologically related to clinical diseases, including anxiety and depression (Elmer and Stadtfeld, 2020; Santini et al., 2020); and cardiovascular diseases (Knox and Uvnäs-Moberg, 1998), among others. The impacts of SIS on the beneficial effects of PA are not completely understood. This way, SIS could confound data interpretation on the health impacts of the running wheel as a rodent model of PA. For instance, SIS prevents the voluntary wheel running-induced proliferation of hippocampal progenitor cells in rats (Leasure and Decker, 2009), suggesting that several benefits of PA may be impacted by sociability conditions.

Although mitochondria play a pivotal role in cells by providing energy for cellular hemostasis and activities, under stress-related conditions, such as SIS, mitochondria may produce excessive amounts of reactive oxygen species, trigger apoptotic pathways, and initiate immune-inflammatory responses. As a consequence, cell damage and death can take place, suggesting that mitochondria could be an important target in the abnormalities induced by SIS (Zhuravliova et al., 2009). Here, we analyzed the effects of free access of mice (socially isolated or grouped) to voluntary wheel running for 21 days on the following: cellular antioxidant defenses (glutathione system); lipid peroxidation index; mitochondrial enzyme activities in the central nervous system tissues, such as cerebellum and cerebral cortex, and peripheral tissues, such as skeletal muscle gastrocnemius, heart, and liver; as well as the mice's behavior.

2. Material and methods

2.1. Animals

Male Swiss mice (8–10 weeks, 30–40 g) were obtained from the animal facility of Federal University of Santa Catarina (UFSC), and housed isolated or in groups of three animals per plastic cage under controlled conditions of light (07:00 to 19:00 h), and temperature (21 ± 1 °C). Mice were allowed to freely access standard laboratory food and tap water. Animals were randomly distributed into experimental groups. All manipulations were carried out between 14:00–17:00 h, being the animals used only once. All procedures in this study were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of the Institution (CEUA PP00282). All efforts were made to minimize animal suffering, and to reduce the number of animals used in the experiments.

2.2. Voluntary wheel running paradigm

Physically active mice had access to homecages (isolated mice 28 × 17 × 13 cm or grouped mice 42 × 34 × 17 cm) containing a running wheel of 12.6 cm in diameter that was attached to the cage, as performed by Cunha et al. (2013). The control group (sedentary) was placed in cages lacking the running wheel. On the 21st experimental day, physically active animals were removed from their homecages, and housed for a period of 24 h in a new cage, without access to the running wheel. Animals were subsequently euthanized by decapitation and cerebral cortex, cerebellum, heart, skeletal muscle gastrocnemius and liver were dissected for biochemical analyses. Using another set of animals the same PA protocol was applied. Subsequently, mice were housed in a new cage without the running wheel for a period of 24 h before exposure to the open field, and, 1 h later, to the tail suspension test (TST).

2.3. Evaluation of the distance travelled and time of activity in the running wheel

The distance travelled and time spent on the running wheel were measured through a magnetic counter attached to the running wheel. These parameters were counted in the cages containing 1 or 3 animals. In the cages containing 3 animals, one third of the values obtained in the magnetic counter was used, corresponding to the average PA for each animal.

2.4. Tissue preparation

For the analyses of oxidative stress-related parameters, the cerebral cortex, cerebellum, heart, skeletal muscle gastrocnemius and liver were obtained. The analyses included nonprotein thiol groups (NPSH), thiobarbituric acid reactive species (TBARS), and glutathione peroxidase (GPx) and glutathione reductase (GR) activities. The tissues were homogenized (1:10 w/v) in phosphate buffer (50 mM, pH 7.4), and the homogenates centrifuged at 16,000 ×*g*, 4 °C for 20 min. The supernatants were used for the determination of GPx and GR activities and to quantify the levels of NPSH and TBARS.

For the activities of mitochondrial complexes I and II, the gastrocnemius muscle and the cerebral cortex were homogenized in 20 volumes of 50 mM potassium phosphate buffer (pH 7.4), containing 0.3 M sucrose, 5 mM 3-(N-morpholino)propanesulfonic acid (MOPS), 1 mM egtazic acid (EGTA), and 0.1% bovine serum albumin. The homogenates were centrifuged at 1000 *g* for 10 min at 4 °C. The pellet containing nuclei and cell debris was discarded and the resulting supernatants, containing a suspension of preserved organelles, including mitochondria, were kept at −70 °C until enzyme activity was determined. The maximal period between homogenate preparation and enzyme activity measurement was < 5 days.

2.5. Non-protein thiol levels measurement

The determination of NPSH was followed according to the method of Ellman (1959), with slight modifications (de Oliveira et al., 2013). An aliquot of tissue homogenate was supplemented on ice with 10% trichloroacetic acid (TCA), mixed, and centrifuged at 15,000*g* for 5 min. The supernatant was used for NPSH determination. The reaction media contained 800 mM sodium phosphate buffer (pH 7.4), and 5 mM 5,50-dithiobis-2-nitrobenzoic acid (DTNB). After sample addition, the color

development, resulting from the reaction between DTNB and thiols, reaches a maximum within 5 min, and it is stable for > 30 min. The absorbance was determined at 412 nm after 10 min. Results were calculated as nanomoles of NPSH per milligram of protein. The DTNB molar extinction coefficient (ε) was 13.6 × 10^3 M^{-1} cm^{-1}.

2.6. TBARS levels analysis

The thiobarbituric acid reactive species (TBARS) assay is probably the oldest and one of the most widely used methods evaluating malondialdehyde (MDA), as an index of lipid peroxidation. TBARS levels were determined in tissue homogenates as described by Ohkawa et al. (1979), in which MDA forms an adduct with two thiobarbituric acid molecules, producing a pink color species that strongly absorbs at 532–535 nm (Dasgupta and Klein, 2014). Briefly, the samples were incubated at ~100 °C for 60 min in acetic acid buffer containing 0.45% sodium dodecyl sulfate, and 0.6% thiobarbituric acid. After centrifugation, the reaction product was determined at 532 nm, based on a standard curve made of a known concentration of 1,1,3,3-tetramethoxypropane. The results were presented as nmol/mg protein.

2.7. Glutathione peroxidase (GPx) activity evaluation

The activity of GPx was evaluated by the method described by Wendel (1981), where the reaction medium contained 0.1 M potassium phosphate buffer (pH 7.0), 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM GSH, 0.225 mM nicotinamide adenine dinucleotide phosphate (NADPH), and 0.2 U/ml GR. The reaction was started by the addition of cumene peroxide (CHP) to a final concentration of 1.0 mM. The GPx enzyme present in the sample reduces CHP using GSH as the electron donor, which results in CHP alcohol, and oxidized glutathione (GSSG). The GSSG formed was rapidly reduced by the added GR with the proportional consumption of NADPH, which can be measured spectrophotometrically at 340 nm.

2.8. Glutathione reductase (GR) activity assay

The GR activity was measured by the method of Carlberg and Mannervik (1985), where the reaction medium contained 0.1 M phosphate buffer, pH 7.0, 1 mM EDTA, and 0.225 mM NADPH. When adding 1 mM GSSG to the reaction media, the GR present in the sample reduces GSSG to GSH, thereby consuming NADPH. This NADPH consumption can be quantified by reading the absorbance at 340 nm.

2.9. Measurement of the respiratory chain enzyme activities

The activity of mitochondrial complex I was determined spectrophotometrically according to Cassina and Radi (1996), and adapted by our group (Remor et al., 2019). The tissue homogenates were added to the reaction media containing 0.2 mM NADH, 0.5 mM ferricyanide, and 5 mM rotenone. The rate of reduced nicotinamide adenine dinucleotide (NADH)-dependent ferricyanide reduction was followed at 420 nm (30 °C, ε = 1 mM^{-1} cm^{-1}).

The activity of succinate:cytochrome *c* oxidoreductase (complex II-CoQ-complex III) was obtained according to Fischer et al. (1985), and slightly modified, as detailed in a previous report (Latini et al., 2005). Before use, the homogenates were submitted to 3 cycles of freezing and thawing. The complex II was activated by a pre-incubation medium (50 mM potassium phosphate buffer (pH 7.4), 20 mM sodium succinate, and 10 pM 2,6-dichlorophenolindophenol (DCPIP)), for 20 min, at 37 °C. The complex II activity was estimated at a final concentration of 50 pM DCPIP, 2 mM potassium cyanide, 2 pg/ml rotenone, and 2.5 mM sodium azide. Enzymatic activity was observed at 600 nm (25 °C, ε = 19.1 mM^{-1} cm^{-1}) in a microplate reader Infinite M200 TECAN. The complex II activity was calculated from the initial change in absorbance.

The activities of the respiratory chain complexes were calculated as nmol/min/mg protein.

2.10. Protein measures

Protein content was estimated as previously described by Bradford (1976), using bovine serum albumin as a standard.

2.11. Behavior tests

2.11.1. Tail suspension test

The TST has become one of the most widely used tests for assessing antidepressant-like activity in mice. The test is based on the fact that animals subjected to the short-term inescapable stress of being suspended by their tail will develop an immobile posture. The total duration of immobility, induced by the tail suspension, was measured according to the original method (Steru et al., 1985). Mice, both acoustically and visually isolated, were suspended 50 cm above the floor by adhesive tape that was placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered immobile only when they hung passively and completely motionless (Cunha et al., 2008).

2.11.2. Open field test

To assess the possible effects of PA and/or SIS on locomotor and exploratory activity, mice were evaluated in the open-field paradigm as previously described (Cunha et al., 2008). Mice were individually placed in a wooden box (40 × 60 × 50 cm) with the floor divided into 12 equal rectangles. The number of rectangles crossed by the animal with its four paws (crossing), and the rising of the front paws (rearing), were registered during a period of 6 min. The number of crossings was considered as indicative of locomotor activity, and the number of rearings was considered as the exploratory activity.

2.12. Statistical analysis

Data were presented as means + S.E.M. (standard error of the mean). Pairwise comparisons between experimental and control groups were performed by Student's *t*-test, otherwise, two-way analysis of variance (ANOVA) was followed by Newman-Keuls post-hoc test, when appropriate. The two investigated factors were sociability (isolated x grouped) and voluntary wheel running exposition (sedentary x PA). A Pearson's correlation analysis was performed to identify any possible relationship between the oxidative stress-related parameters and the distance travelled, or time spent on the voluntary wheel running. A value of $P < 0.05$ was considered significant.

3. Results

3.1. The effects of social isolation on the activity parameters in the running wheel

Socially isolated mice travelled greater distance in the running wheel, as compared to grouped animals (Fig. 1A). Furthermore, socially isolated mice spent longer time on the running wheel, as compared to grouped animals (Fig. 1B). The student's *t*-test revealed significant differences between experimental groups for the travelled distance and for the time spent on the running wheel, as evaluated by the area under the curve ($t(10) = 2.02$; $p < 0.05$; and $t(10) = 2.24$; $p < 0.05$, respectively).

3.2. Lipid peroxidation in the socially isolated or grouped mice exposed to running wheel

In the present study we also investigated the effect of free access of animals to voluntary wheel running on a lipoperoxidation index,

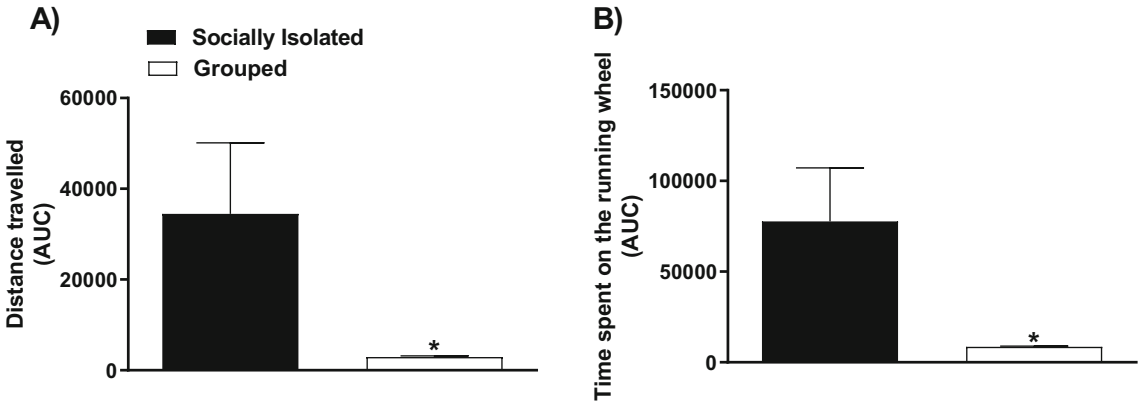

Fig. 1. The effect of housing conditions (socially isolated or grouped animals) on the distance travelled and time spent during the voluntary physical activity. The effect of housing conditions on the area under the curve of the distance travelled (Panel A) or the time spent on the running wheel (Panel B) over 21 days. Data are presented as mean of the area under curve of six mice/group. $^{**}p < 0.01$ as compared to sedentary socially isolated mice (Student's *t*-test).

TBARS. We evaluated the TBARS levels in the cerebral cortex, cerebellum, heart, skeletal muscle and liver from socially isolated or grouped animals and/or exposed to running wheel (Fig. 2A-E). Socially isolated mice presented higher TBARS levels in the cerebral cortex, as compared to grouped animals, and voluntary PA in the running wheel was unable to reverse this alteration. The two-way ANOVA showed a significant effect for sociability ($F(1,20) = 7.313$; $p < 0.05$), but not for PA and sociability × PA interaction.

Interestingly, the socially isolated mice exposed to the running wheel presented lower TBARS levels in the gastrocnemius, as compared to sedentary socially isolated mice (Fig. 2D). The two-way ANOVA showed a significant effect for sociability ($F(1,20) = 17.41$; $p < 0.01$), and sociability × PA interaction ($F(1,20) = 8.59$; $p < 0.01$), but not for PA.

Additionally, animals in the presence of running wheel presented decreased TBARS levels in the liver of grouped animals, as compared to sedentary grouped animals (Fig. 2E). The two-way ANOVA showed a significant effect for sociability ($F(1,20) = 5.50$; $p < 0.05$), PA ($F(1,20) = 4.78$; $p < 0.05$), and sociability × PA interaction ($F(1,20) = 5.03$; $p < 0.05$). The two-way ANOVA showed no significant changes in TBARS levels in the heart and skeletal muscle.

3.3. The effects of running wheel on NPSH levels of socially isolated or grouped mice

In the present study we also investigated the effect of free access of animals to voluntary wheel running on NPSH levels, being 95% comprised of GSH (Heisinger and Wait, 1989; DeLucia et al., 1975; Cohn and Lyle, 1964). NPSH was evaluated in the cerebral cortex, cerebellum, heart, skeletal muscle, and liver from socially isolated or grouped animals and/or exposed to running wheel (Fig. 3A-E). The main effect revealed significant differences for NPSH levels in the gastrocnemius between socially isolated and grouped mice (Fig. 3D). The two way ANOVA showed a significant effect for sociability ($F(1,20) = 9.59$; $p < 0.05$), but not for PA ($F(1,20) = 0.045$; $p = 0.84$), or sociability × PA interaction ($F(1,20) = 0.76$; $p = 0.39$).

The results depicted in the Fig. 3A showed that PA protocol leads to increased NPSH levels in the cerebral cortex of grouped mice, as compared to sedentary group. The two-way ANOVA showed a significant effect for sociability ($F(1,20) = 12.04$; $p < 0.01$), PA ($F(1,20) = 4.87$; $p < 0.05$), and sociability × PA interaction ($F(1,20) = 4.76$; $p < 0.05$). However, we did not observed alterations in the NPSH levels in other body tissues analyzed following SIS and/or PA paradigm.

3.4. The effect of running wheel on the activity of GPx and GR in distinct body tissues of socially isolated or grouped mice

In the present study we also investigated the effect of PA paradigm induced by free access of mice to running wheel on the activity of GPx and GR enzymes, as evaluated in the cerebral cortex, cerebellum, heart, skeletal muscle and liver (Fig. 4A-J) of socially isolated or grouped mice.

Among the sedentary animals, grouped mice presented lower GPx and GR activities in the cerebellum, as shown in Fig. 4C [sociability ($F(1,20) = 8.23$; $p < 0.05$), PA($F(1,20) = 7.34$; $p < 0.05$), and sociability × PA interaction ($F(1,20) = 13.12$; $p < 0.01$)] and Fig. 4D [sociability ($F(1,20) = 12.41$; $p < 0.05$), PA ($F(1,20) = 3.26$; $p = 0.086$), and sociability × PA interaction ($F(1,20) = 5.54$; $p < 0.05$)]. Furthermore, grouped mice without running wheel access presented lower GPx and GR activities in the gastrocnemius, as shown in Fig. 4G [sociability ($F(1,20) = 5.84$; $p < 0.05$), PA ($F(1,20) = 3.08$; $p = 0.09$), and sociability × PA interaction ($F(1,20) = 1.19$; $p = 0.29$)], and Fig. 4H [sociability ($F(1,17) = 0.04$; $p = 0.064$), PA ($F(1,17) = 1.30$; $p = 0.36$), and sociability × PA interaction ($F(1,17) = 4.0011$; $p < 0.01$)]. Additionaly, grouped mice presented lower GR activity in the liver, as shown in Fig. 4J [sociability ($F(1,20) = 4.76$; $p < 0.05$), PA ($F(1,20) = 0.22$; $p = 0.65$), and sociability × PA interaction ($F(1,20) = 15.24$; $p < 0.05$)].

PA protocol decreased GPx and GR activities in the cerebral cortex of socially isolated animals, as shown in Fig. 4A [sociability ($F(1,20) = 31.15$; $p < 0.05$), PA ($F(1,20) = 5.04$; $p < 0.05$), and sociability × PA interaction ($F(1,20) = 3.69$; $p = 0.099$)], and Fig. 4B [sociability ($F(1,20) = 12.41$; $p < 0.05$), PA ($F(1,20) = 3.26$; $p = 0.086$), and sociability × PA interaction ($F(1,20) = 5.54$; $p < 0.05$)]. PA protocol also decreased GPx activity in the heart of mice, as shown in Fig. 4E [sociability ($F(1,20) = 1.46$; $p = 0.24$), PA ($F(1,20) = 5.23$; $p < 0.05$), and sociability × PA interaction ($F(1,19) = 0.11$; $p = 0.75$)]. Mice that were socially isolated and exposed to running wheel presented decreased GR activity in the liver, as shown in Fig. 4J. In contrast, mice submitted to PA presented increased GPx activity in the cerebellum of grouped animals, as shown in Fig. 4C.

3.5. The effect of running wheel on the activity of mitochondrial complexes I and II in cerebral cortex and gastrocnemius muscle of socially isolated or grouped mice

In the present study we also investigated the effect of free access of mice to the voluntary wheel running on the activity of mitochondrial complexes I and II of the electron transport chain in the cerebral cortex and skeletal muscle gastrocnemius (Fig. 5A-D) of socially isolated or

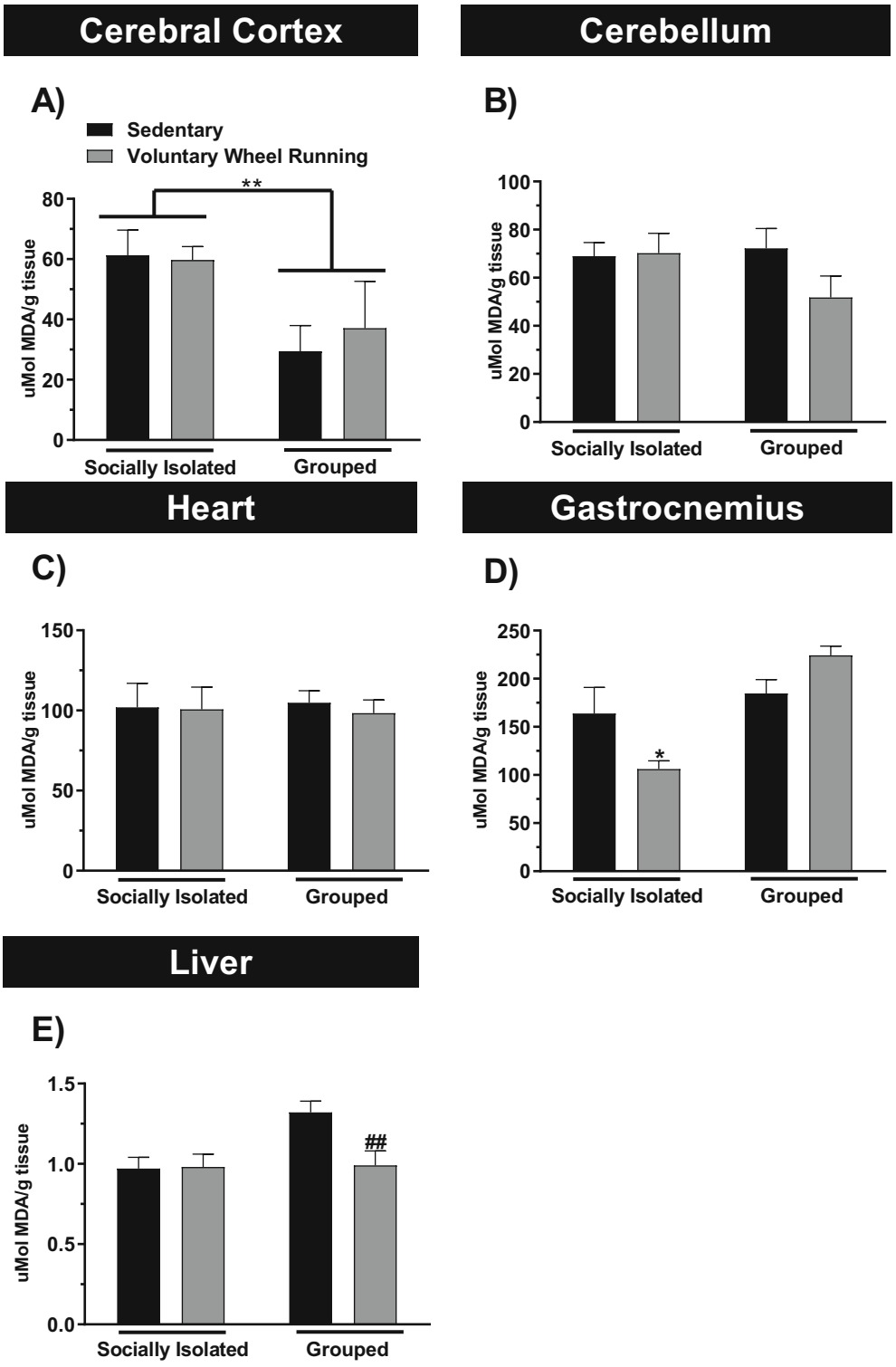

Fig. 2. The effect of physical activity and/or chronic social isolation on the lipoperoxidation index. The effect of physical activity on the TBARS levels in the cerebral cortex (Panel A), cerebellum (Panel B), heart (Panel C), gastrocnemius (Panel D) and liver (Panel E) of the socially isolated or grouped mice. Data are presented as mean + SEM of six mice/group. $^{*}p < 0.05$, $^{**}p < 0.01$ as compared to sedentary socially isolated mice. $^{\#}p < 0.05$ as compared to sedentary grouped mice (two-way ANOVA followed by Newman-Keuls post-hoc test).

grouped animals. The results showed that the individualized animals had a higher activity of mitochondrial complexes I and II in the cerebral cortex, as shown in Fig. 5A [sociability ($F(1,20) = 7.04$; $p < 0.05$), but not for PA ($F(1,20) = 0.13$; $p = 0.73$), or sociability × PA interaction ($F(1,20) = 2.17$; $p = 0.16$)], and Fig. 5B [sociability ($F(1,20) = 4.70$; $p < 0.05$), PA ($F(1,20) = 1.17$; $p = 0.29$), and sociability × PA interaction ($F(1,20) = 4.82$; $p < 0.05$)]. The PA did not alter the activities of mitochondrial complexes I and II in the socially isolated or grouped animals.

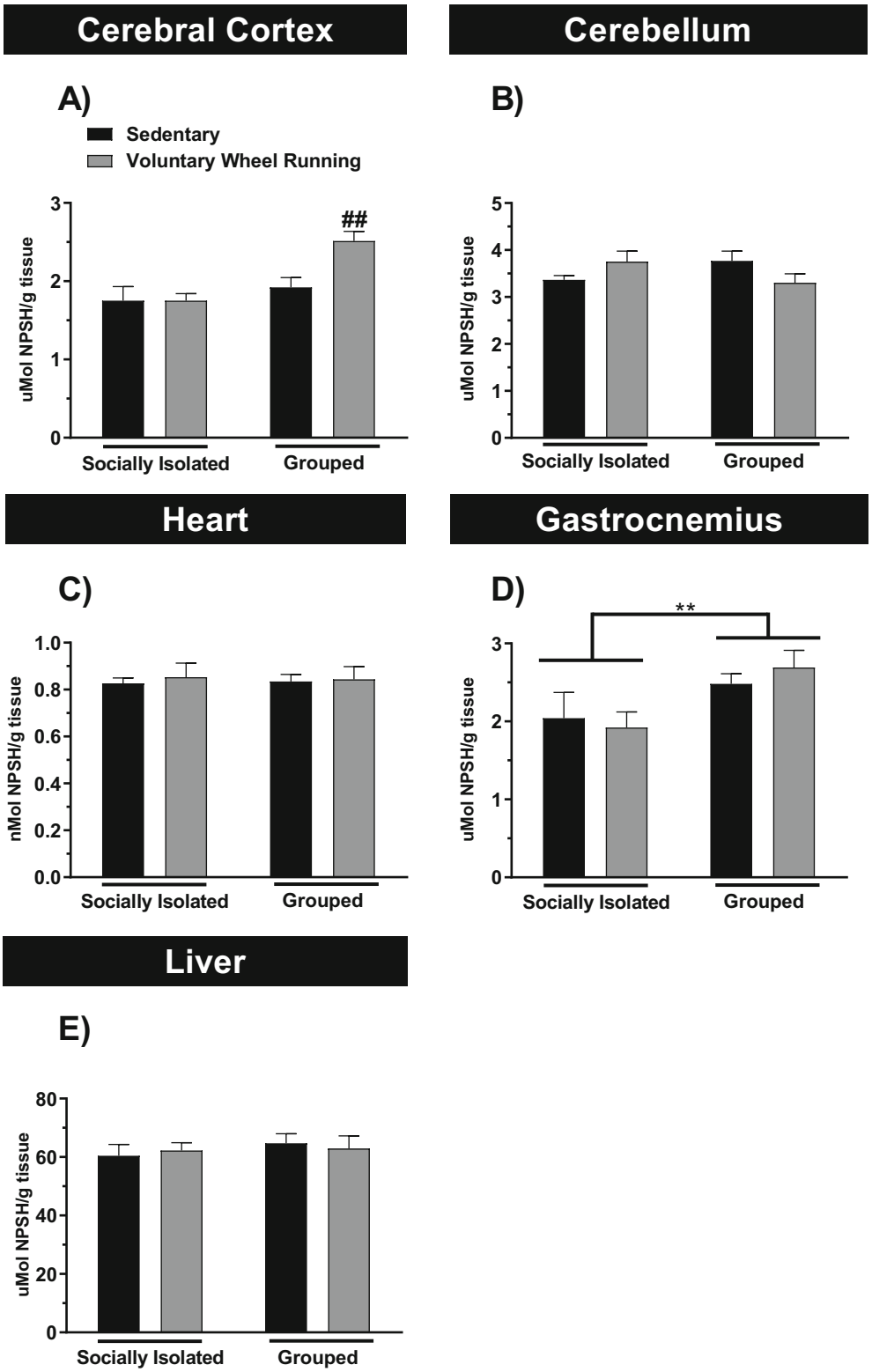

Fig. 3. The effect of physical activity and/or chronic social isolation on the non-protein thiols (NPSH) levels. The effect of physical activity on the NPSH levels in the cerebral cortex (Panel A), cerebellum (Panel B), heart (Panel C), gastrocnemius (Panel D) and liver (Panel E) of the socially isolated or grouped mice. Data are presented as mean + SEM of six mice/group. $^{**}p < 0.01$ as compared to sedentary socially isolated mice (two-way ANOVA followed by Newman-Keuls post-hoc test).

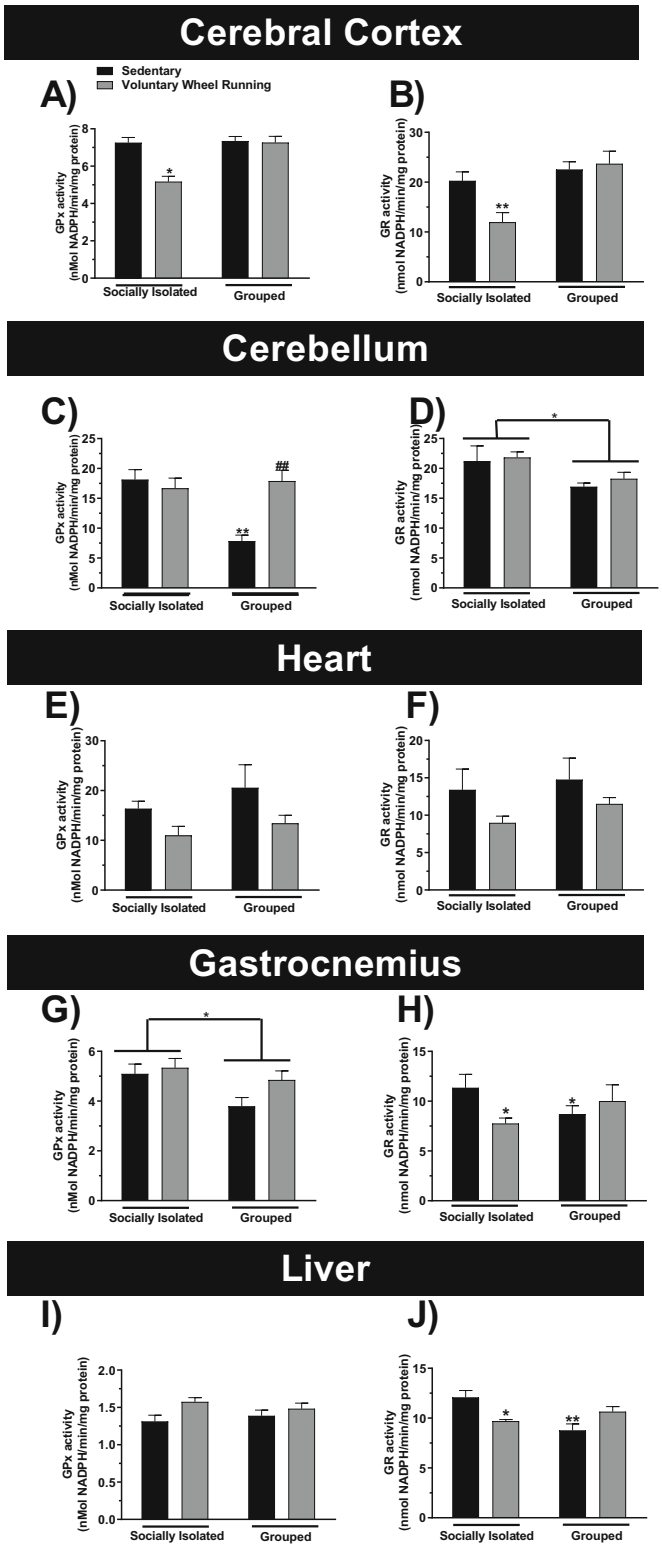

Fig. 4. Physical activity and/or chronic social isolation induce alterations on the glutathione peroxidase (GPx) or glutathione reductase (GR) activities. The effect of physical activity on the GPx or GR activities in the cerebral cortex (Panels A and B, respectively), cerebellum (Panels C and D, respectively), heart (Panels E and F, respectively), gastrocnemius (Panels G and H, respectively), liver (Panels I and J, respectively) of the socially isolated or grouped mice. $^{*}p < 0.05$, $^{**}p < 0.01$ as compared to sedentary socially isolated mice. $^{\#}p < 0.05$ as compared to sedentary grouped mice ($n = 6$; two-way ANOVA followed by Newman-Keuls post-hoc test).

3.6. The effect of running wheel on the immobility time in the tail suspension test and the behavior in the open field of mice socially isolated or grouped mice

We also evaluated the effect of free access of mice to the running wheel on the immobility time in the TST of socially isolated or grouped animals. The results showed that both, individualized and grouped animals exposed to the running wheel, presented lower immobility time in the TST, as compared to respective control groups (Fig. 6A). A two-way ANOVA showed significant differences for PA ($F(1,32) = 12.20$, $P < 0.05$), but not for sociability ($F(1,32) = 0.027$, $p = 0.87$) and PA × sociability interaction ($F(1,32) = 0.012$, $p = 0.91$).

The ambulation and exploratory behavior in the open field were also investigated in the present study. The number of crossings and rearings in the open field was not altered by free access to the running wheel (Fig. 6B-C).

3.7. Analysis of correlation between oxidative stress-related parameters or immobility time in the TST and distance travelled or time spent on the running wheel

Fig. 7 depicts the significant correlations between the oxidative stress-related parameters or behavior and the distance travelled or time spent on the running wheel. The Pearson's test showed a positive correlation between MDA levels in the cerebral cortex with the distance travelled and the time spent on the running wheel ($P < 0.05$, Fig. 7A-B). The distance travelled and the time spent on the running wheel tended to be correlated with MDA levels in the cerebellum (Fig. 7C-D). Furthermore, NPSH levels did not correlated with PA parameters ($P > 0.05$, data not shown).

The GPx activity in the cerebellum was negatively correlated with the distance travelled and the time spent on the running wheel ($P < 0.05$, Fig. 7E-F). Furthermore, GPx activity in the heart was correlated with the distance travelled in the running wheel ($P < 0.05$, Fig. 7G) and tended to correlate with the time spent on the running wheel (Fig. 7H). The Pearson's test also showed a positive correlation of GR activity in the gastrocnemius muscle with the distance travelled and the time spent on the running wheel ($P < 0.05$, Fig. 7I-J).

The complex I and II activities in the cerebral cortex and gastrocnemius muscle did not correlate with the distance travelled and the time spent on the voluntary wheel running ($P > 0.05$, data not shown).

Finally, the immobility time in the TST was negatively correlated with the distance travelled and the time spent on the running wheel ($P < 0.05$, Fig. 7K-L). Furthermore, the number of crossings and rearings in the open field did not correlate with the distance travelled and the time spent on the voluntary wheel running ($P > 0.05$, data not shown).

4. Discussion

Social isolation stress (SIS) profoundly affects the daily activity of individuals and is considered an important risk component for morbidity and mortality in humans (Cacioppo et al., 2015). In particular, the COVID-19 pandemic has brought the impact of SIS to the world in the last months (Oliveira and Rossi, 2020). Imposed isolation by the COVID-19 pandemic is a unifamiliar and unpleasant experience that involves separation from friends and family, and a departure from usual, everyday routines (Onyeaka et al., 2020). In line with this, studies indicate that SIS in rodents is a stressful paradigm, since it produces a negative impact on behavior and endocrine responses, such as increased corticosterone, adrenocorticotropic hormone (ACTH), and catecholamine serum levels (Grippo et al., 2007a, 2007b, 2007c; Weiss et al., 2004). The results of a systematic review suggested that PA interventions are associated with decreased SIS among community-dwelling older adults, suggesting an important protective role of PA on

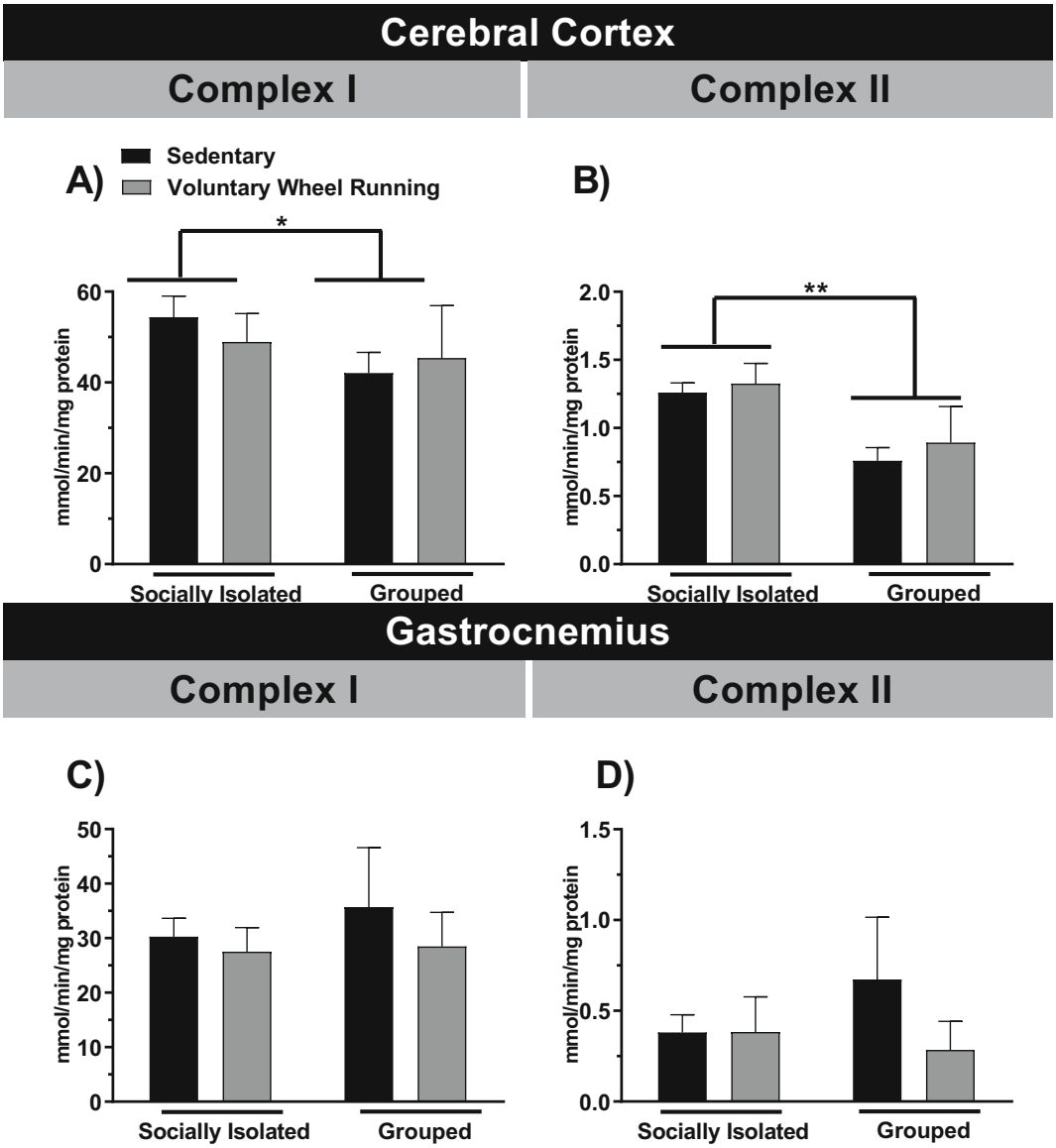

Fig. 5. The effect of physical activity and/or chronic social isolation on the complex I and II activities of mice. The effect of physical activity on the complex I or II activities in the cerebral cortex (Panels A and B, respectively) and gastrocnemius (Panels C and D, respectively), of the socially isolated or grouped mice. *p < 0.05 and **p < 0.01 as compared to sedentary socially isolated mice (n = 5–6; two-way ANOVA followed by Newman-Keuls post-hoc test).

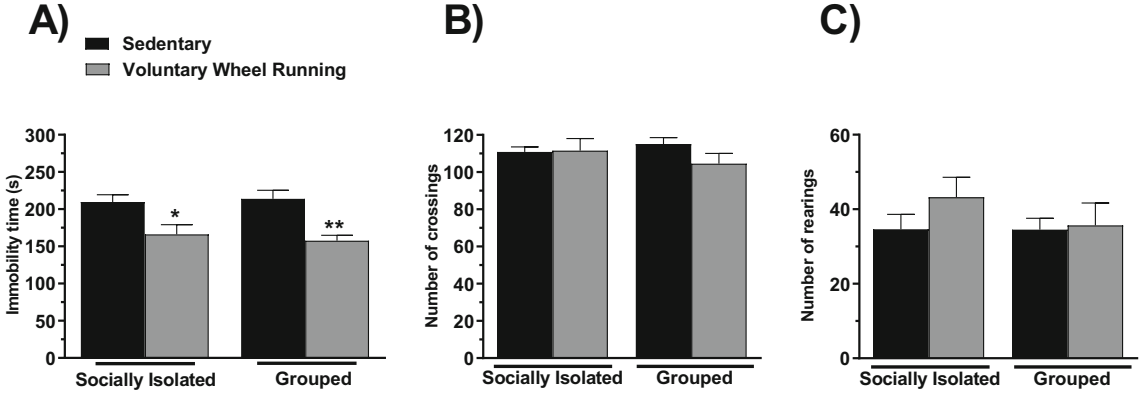

Fig. 6. Voluntary wheel running induces antidepressant-like effect in the socially isolated and grouped animals. The effect of physical activity on the immobility time in the TST (Panel A) and the crossings and rearings in the open field test (Panels B and C, respectively) of the socially isolated or grouped mice. *p < 0.05 and **p < 0.01 as compared to sedentary socially isolated mice (n = 9; two-way ANOVA followed by Newman-Keuls post-hoc test).

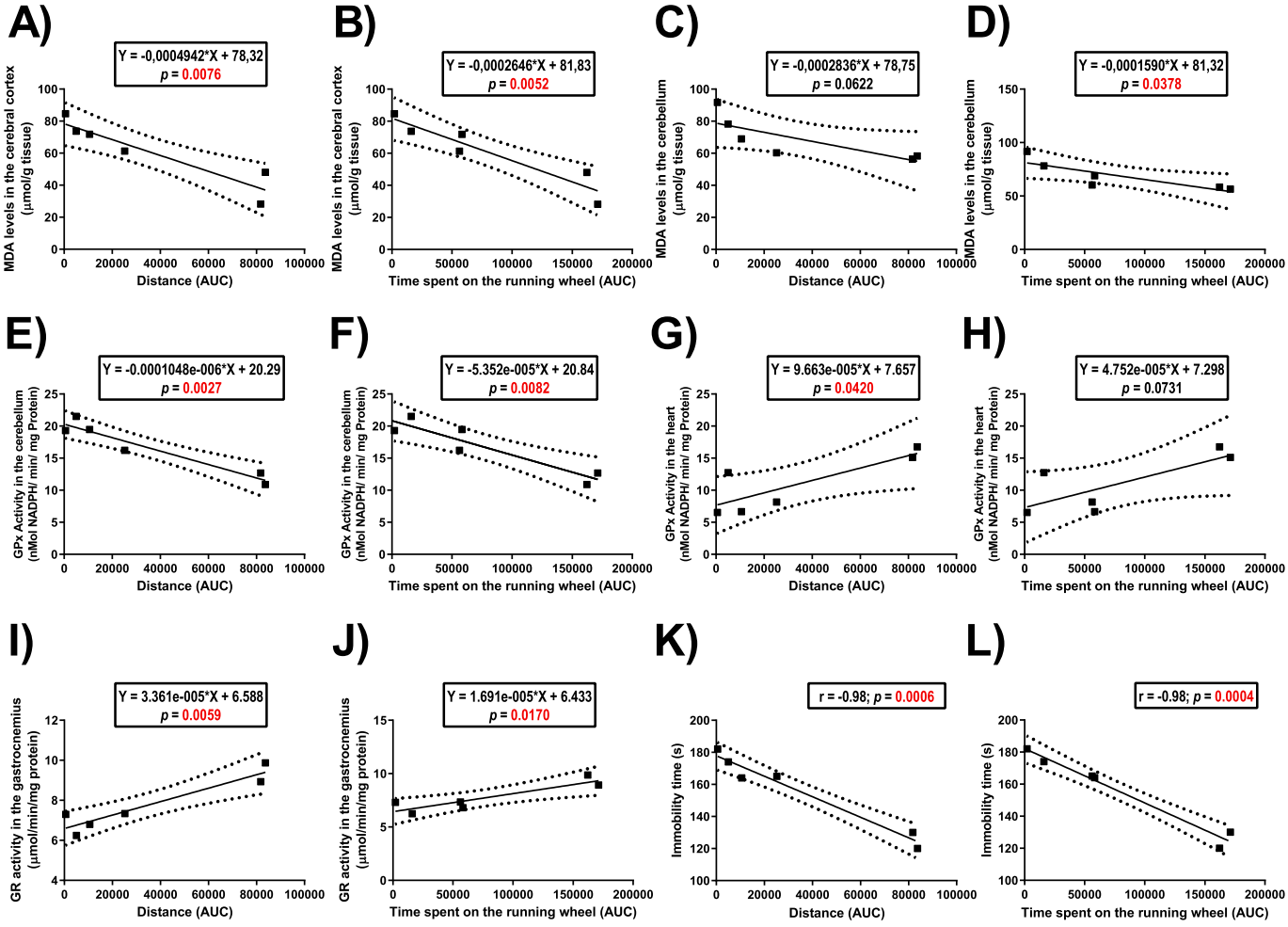

Fig. 7. Correlation between the oxidative stress-related parameters or behavior and the distance travelled or time spent on the running wheel of socially isolated mice. The correlation between distance travelled or time spent on the voluntary wheel running with: 1) TBARS levels in the cerebral cortex (Panels A and B), and cerebellum (Panels C and D); 2) GPx activity in the cerebellum (Panels E and F) and heart (Panels G and H); 3) GR activity in the gastrocnemius (Panels G and H); and the immobility time in the tail suspension test (Panels I and J).

the negative effects of SIS (Robins et al., 2018). Many activity/exercise programs have been designed for elderly persons in a bid to reduce isolation and its consequences (Dickens et al., 2011; Landeiro et al., 2017). However, in the present study we showed that grouped animals travelled lower distances in the running wheel, as compared to socially isolated mice. Pitzer et al. (2016a, 2016b) demonstrated that housing conditions (C57BL6 mice individualized or housed in groups of 3 per cage) did not alter the distance travelled in the running wheel (Pitzer et al., 2016a, 2016b). The difference observed in the distance travelled could be explained by distinct mouse strains. In the present study, we used Swiss mice, a strain very sensitive to stressful events, but presenting little running wheel activity compared to other strains such as C57BL6 mice. Interestingly, Swiss mice are more dominant and aggressive than C57BL6 mice (Bisazza, 1979, 1981). The lower distance travelled in the running wheel by grouped Swiss mice, compared to socially isolated mice, could be dependent on the agonistic interaction for dominance and territorialism. Observations, such as those made by Bisazza (1981), suggest that male mice of strains with a high propensity to fight (e.g., Swiss) may greatly benefit from individual housing (Kappel et al., 2017; Poole and Morgan, 1973). Grouped animals tend to fight repeatedly, leading to wounds and to a painful distress condition. Regarding this point, individually housed rats engaged in slightly higher locomotor activity than grouped animals during the active phase (Stranahan et al., 2006). Interestingly, a study reported that CD1 mice, housed in standard polycarbonate cages (5 mice per cage) containing a running wheel, increased their aggressive behavior, disrupting stereotyped behavior and the linearity in dominance hierarchy (Howerton et al., 2008). In the present study, grouped animals without access to the running wheel did not present wounds or signs of distress. However, two mice of the sociability group with free access to the running wheel presented wounds on their backs, suggesting fights between animals may have intensified, which can be restricted to mice exposed to the running wheel. However, body weight change (a stress-related parameter) was not altered by the presence of a running wheel in the grouped or socially isolated mice (data not shown). Similar to our results, studies reported that SIS for 28 days did not alter the body weight of C57BL/6J mice of 12 or 20 weeks (Sun et al., 2014; Ieraci et al., 2016). Further experiments are required to clarify the effect of voluntary wheel running in the homecage of grouped Swiss mice on stress-related parameters and aggressiveness.

Here, we also investigated the impact of SIS on the PA paradigm (voluntary wheel running exposition) effect on oxidative stress-related parameters and mitochondrial function. Specific brain structures (cerebral cortex and cerebellum) and peripheral tissues, such as skeletal muscle gastrocnemius, liver, and heart, were selected for this study. The biochemical analysis in the cerebral cortex is justified due to its implications for cognition and affective behavior, being more sensitive to oxidative stress induced by SIS than other brain tissues such as the hippocampus (Zlatković et al., 2014a). The cerebellum was also a site chosen for biochemical analysis because the cerebellum is critical to

social and affective processing and regulation (Van Overwalle et al., 2014; Hoche et al., 2016; Guell et al., 2018). The peripheral tissues (heart, liver, and skeletal muscle gastrocnemius) were chosen because SIS has been considered a risk factor for cardiovascular disease (Xia and Li, 2018) and overweight with hepatic hypertrophy (Sakakibara et al., 2012). Furthermore, the skeletal muscle was chosen for biochemical determinations because it is a primary tissue target of the PA paradigm using voluntary wheel running (Manzanares et al., 2018).

Chronic stress may disturb redox-status via increased reactive oxygen species, leading to oxidative damage to cellular macromolecules. Oxidative damage can be used as a biomarker of negative health outcomes, usually assessed by measuring lipid peroxidation end products, such as MDA, a reactive aldehyde produced by lipid peroxidation of polyunsaturated fatty acids (Ayala et al., 2014; Sahin and Gümüşlü, 2004). Here, the results suggested that SIS induced lipid peroxidation in the cerebral cortex by 21 days. No changes were observed in the cerebellum, gastrocnemius, and heart of mice. A study performed by Huong et al. (2005) showed that socially isolated mice by 6-8 weeks presented increased TBARS levels in the brain (Huong et al., 2005). Interestingly, socially isolated rats had higher TBARS levels in the prefrontal cortex and hippocampus, compared to socialized rats (Famitafreshi and Karimian, 2018; Zlatković et al., 2014c). It is noteworthy that the cerebellum is a brain structure presenting higher antioxidant capacity and is more resistant to oxidative stress than the cerebral cortex (Latini et al., 2007; Vandresen-Filho et al., 2015). Additionally, we also demonstrated that SIS decreased the TBARS levels in the liver of mice. A study pointed out that rats exposed for 6 weeks to chronic SIS increased cytosolic TBARS levels in the liver (Stanisavljevic et al., 2017). Discrepancies between our results and literature data might be due to the different species used. Furthermore, in the future we intend to carry out experiments using more specific techniques for lipid peroxidation, since TBARS is an unspecific method that is subject to a number of criticisms (Forman et al., 2015). Measuring the levels of isoprostanes and/or MDA-thiobarbituric acid adduct by HPLC would bring more specificity and precision (Forman et al., 2015). Oxidative protein and DNA modifications are also interesting end points to be analyzed in future studies. In line with this, rats that underwent SIS presented higher protein carbonyl levels (Zlatković et al., 2014a) and DNA damage (Krolow et al., 2013) in the brain, suggesting that oxidation of both proteins and DNA could be an important target for SIS.

In the present study, PA intervention using voluntary wheel running was unable to prevent the increase in TBARS levels in the cerebral cortex induced by SIS. Interestingly, free exposition of mice to the running wheel decreased TBARS levels in the liver of grouped mice and in the gastrocnemius skeletal muscle of socially isolated mice. Conversely, rats exposed to voluntary wheel running for 4 weeks did not alter the TBARS levels in the liver, skeletal muscle soleus, and heart (Škop et al., 2015). Similar to our results, Judge et al. (2005) demonstrated the absence of significant differences in lipid peroxidation (4-hydroxy-2-nonenal-modified proteins and TBARS levels) on the hearts of rats physically exercised in the running wheel, compared to sedentary rats (Judge et al., 2005).

Glutathione (GSH) has a pivotal role as an antioxidant defense, either by reacting directly with reactive oxygen and nitrogen species, or by acting as an essential cofactor for the enzymes glutathione S-transferase and GPx (Deponte, 2013). In line with this, thiol groups of GSH serve as electron donors in the reduction of disulfide bonds of cytoplasmic proteins, leading to GSSG build up (Pompella et al., 2003). Since about 85–95% of non-protein sulfhydryl groups (NPSH) are composed of GSH, this method was used as an indirect determination of GSH, and also served as index for the redox status of the cell (Heisinger and Wait, 1989; DeLucia et al., 1975; Cohn and Lyle, 1964). Studies have demonstrated that SIS decreases GSH levels in several tissues. For instance, a study demonstrated that SIS decreased GSH levels in the brain and heart of rats (Sonei et al., 2017). In addition, SIS for 6–8 weeks also depleted brain GSH content in mice (Huong et al., 2005). Chronic SIS also decreased GSH levels in the liver and blood of rats by 21 or 84 days (Mohale and Chandewar, 2012). The present study found statistical differences in NPSH levels on the gastrocnemius, but not on the other tissues, between socially isolated and grouped mice.

The present study is, to the best our knowledge, the first to investigate the effects of the running wheel paradigm on GSH homeostasis, which was investigated in several tissues of socially isolated and grouped mice. The present study showed that the PA paradigm in grouped mice lead to an increase in cerebral cortex NPSH levels compared to the sedentary group, however PA did not alter the NPSH levels in the peripheral tissues analyzed. Similar to our results, GSH levels in the heart were not altered following the running wheel paradigm (Judge et al., 2005). Contrasting to these results, the levels of GSH in the liver of physically active male and female rats were greater than the sedentary group (Yamamoto et al., 2002). These dissimilar responses regarding hepatic GSH levels could be due to different animal strains/species or differences in the experimental protocol. We intend to further investigate how GSH levels are relevant to SIS in the context of PA in future studies.

It is known that GSH is a cofactor for GPx in the reduction of lipid hydroperoxides to their corresponding alcohols, as well as in the reduction of hydrogen peroxide to water, releasing GSSG as the by-product (Bhabak and Mugesh, 2010; Flohe et al., 1973). GR then reduces GSSG, regenerating GSH to complete the catalytic cycle (Deponte, 2013). In the present study, SIS increased GPx activity in the cerebellum and gastrocnemius muscle, while GR activity was increased in the cerebellum, gastrocnemius muscle, and liver. Studies are controversial in relation to how SIS modulates the GSH system. For instance, SIS by 21 days impaired GSH-dependent protection, since the stress paradigm decreased GSH levels and increased GPx activity and immunocontent in the rat prefrontal cortex (Todorović and Filipović, 2017a, 2017b; Zlatković et al., 2014a). Furthermore, chronic SIS decreased the activity, but not the immunocontent, of GPx in the hippocampus of rats (Djordjevic et al., 2010). Chronic SIS decreased GSH content and GR activity in the liver of rats, but GPx activity remained unaltered (Todorović et al., 2016).

The PA protocol abrogated the increase in GPx and GR activities induced by SIS in the peripheral tissues, such as the gastrocnemius and liver. Additionally, we showed that PA exposition decreased GPx and GR activities in the cerebral cortex of socially isolated animals, suggesting a central nervous system adaptation to the PA paradigm. In the present study, the PA protocol did not alter the heart, GPx, or GR activities. Similar to our results, a study showed that the running wheel paradigm does not alter GSH levels, or GPx and GR activities in the heart (Judge et al., 2005). Additionally, rats exposed to the running wheel for 4 weeks did not have altered GPx activity in the liver, skeletal muscle soleus, or heart (Škop et al., 2015).

Emerging evidence suggests that mitochondrial function is an early target of stress (Gardner and Boles, 2005, 2008; Jeanneteau et al., 2018; Picard et al., 2018; Ridout et al., 2016). Mitochondria are ubiquitous organelles in eukaryotic cells responsible for orchestrating cellular energy production in the form of ATP via protein complexes mediating oxidative phosphorylation. The NADH ubiquinone oxidoreductase (complex I) is the first enzyme of the respiratory chain. It oxidizes NADH, which is generated through the Krebs cycle in the mitochondrial matrix, and uses the two electrons to reduce ubiquinone to ubiquinol. The succinate ubiquinone oxidoreductase (complex II) catalyzes the oxidation of succinate to fumarate and the reduction of ubiquinone to ubiquinol. These two enzyme complexes are the gateway to oxidative phosphorylation, and can serve as indicators of mitochondrial function. Furthermore, previous studies demonstrated the pivotal role of these mitochondrial complexes in social behavior (Hollis et al., 2015). Interestingly, the cerebral cortex is more sensitive than the hippocampus to the effects of SIS with regards to mitochondrial function, taking into account that: 1) cerebral cortex, but not hippocampus, of rats exposed

to SIS by 21 days presented increased cytochrome *c* release from mitochondria into the cytosol (Filipović et al., 2011); and 2) SIS did not alter the activity of both respiratory chain complexes I/III and II in the hippocampus of juvenile rats (Krolow et al., 2012). For this reason, we performed activity analyses of mitochondrial complexes in the cerebral cortex of animals. Furthermore, the skeletal muscle gastrocnemius was another tissue chosen to have its mitochondrial complex activity analyzed, based on the peripheral and direct association with voluntary wheel running, as this protocol stimulates its contraction. We did not analyze the enzymatic activity of mitochondria complexes in cardiac tissue because studies have reported that rats exposed to lifelong running wheel had displayed no effects on aerobic respiration parameters, such as the rate of oxygen consumption (state 4 or state 3) or respiratory control ratio, in the heart tissue (Servais et al., 2003; Judge et al., 2005).

In the present study, we demonstrated that socially isolated mice increased the activity of both complex I and II in the cerebral cortex, but not in the skeletal muscle gastrocnemius. However, studies indicated that SIS could be associated with impaired respiratory chain complex II, which can lead to reactive oxygen species formation, oxidative damage, and ATP depletion in both the brain and heart of rats (Sonei et al., 2017; Zhuravliova et al., 2009). The differences in the activity of complex II found in the present study in relation to literature data could be associated with species, age, and SIS protocol. Although there are no reports investigating the effects of free exposure of rodents to the running wheel on changes in the mitochondrial function induced by SIS, there is evidence indicating that the running wheel paradigm could modulate mitochondrial activity (Herbst and Holloway, 2015; Marques-Aleixo et al., 2015; Stolle et al., 2018). In the present study, we demonstrated that individually housed animals presented higher activity of mitochondrial complexes I and II in the cerebral cortex, but not in the gastrocnemius muscle. The PA paradigm was unable to alter the activity of mitochondrial complexes I and II. Interestingly, isolated brain mitochondria of C57BL/6 mice submitted to SIS, in combination with running wheel for 6 weeks, presented increased mitochondrial complex I activity and abrogated the inhibition of this complex induced by rotenone incubation, while the effects of MPP^+ were not altered (Aguiar et al., 2014; Aguiar Jr et al., 2014). Furthermore, rats exposed to voluntary wheel running increased the activities of complex I and II in the soleus skeletal muscle (Hedges et al., 2019). The differences in the activities of mitochondrial complexes could be associated with differences in the strain and species.

Given that stress is the main triggering factor of major depressive disorder, we investigated the effect of SIS and PA on the immobility time in the TST. The results showed that the PA protocol produced a reduction in the immobility time of mice submitted to the TST, an antidepressant-like profile. The antidepressant-like effect of the voluntary wheel running paradigm has previously been shown (Cunha et al., 2013; Duman et al., 2008). However, under the effect of SIS, the antidepressant-like effect may limit the interpretation of data, precluding further discussions and conclusions. In the present study, the behavior of mice in the TST was not dependent of social condition, since SIS was unable to alter the immobility time. Similar to our results, rats submitted to SIS for 1, 2, or 3 weeks did not present depressive-like effects in the forced swimming test (FST; Gorlova et al., 2018). Furthermore, a study showed that SIS increased the immobility time of Wistar rats in the FST, which was not observed for Wistar-Kyoto rats, suggesting that the effect of SIS can be strain-specific (Mileva and Bielajew, 2015). Interestingly, a study reported that predisposed Wistar rats presenting a background of increased immobility time in the FST presented a depressive-like profile when submitted to SIS for 21 days. The same effect was not observed in non-predisposed Wistar rats, suggesting that predisposition to the effects of stress is a determining factor for behavior despair following SIS (Zanier-Gomes et al., 2015). Moreover, several reports also revealed that SIS significantly increased the immobility time of NMRI and C57BL/6J mice or Sprague-Dawley rats submitted to the FST (Chan et al., 2017; Cho et al., 2017; Dávila-Hernández et al., 2018; Haj-Mirzaian et al., 2015, 2019). The discrepancies of our results in the behavior despair test, compared to the literature data, could be due to several factors, including biological factors such as strain, age, body weight, gender, and individual differences between animals.

We also investigated the correlation between distance travelled, or time spent on the running wheel, and oxidative stress-related parameters, as well as the mitochondrial function in the socially isolated mice. The TBARS levels were negatively correlated with the distance travelled and the time spent on the running wheel. Interestingly, a tendency of negative correlation between cerebellar TBARS levels and distance travelled or time spent on the running wheel was pointed out in the present study. These data suggest that PA could be protective against lipid peroxidation induced by SIS. Furthermore, the NPSH levels did not correlate with the distance travelled or the time spent on the running wheel. However, the literature data reported that GSH levels of the liver and brain were correlated with distance travelled in the running wheel (Yamamoto et al., 2002). Additionally, the present results also demonstrated that cerebellar GPx activity was negatively correlated with the distance travelled, or the time spent on the running wheel. Moreover, the heart GPx activity was positively correlated with the distance travelled. GR activity in the gastrocnemius was also positively correlated with distance travelled or time spent on the running wheel. These data indicated that PA could modulate the GSH-dependent enzymatic antioxidant system. Furthermore, a negative correlation between the immobility time in the FST and distance travelled and the time spent on the running wheel was reported in the present study, suggesting that PA was an important tool for clinical depression management.

5. Conclusion

Collectively, our results demonstrate that SIS produces mitochondrial functional alterations and oxidative stress, since it increases lipid peroxidation and alters the activity of the antioxidant enzymatic system dependent on GSH in peripheral and central tissues. Therefore, we further showed that a voluntary wheel running-induced decrease in oxidative stress is dependent, at least in part, on housing conditions. In

Table 1
Summary of the main neurochemical findings in mice following social isolation and/or physical activity paradigm in the running wheel.

	Cerebral Cortex		Cerebellum		Heart		Gastrocnemius		Liver	
	Social isolation	Running wheel	Social isolation	Running wheel	Social isolation	Running wheel	Social isolation	Running wheel	Social isolation	Running wheel
MDA	↑	–	–	–	–	–	–	↓	–	↓
NPSH	–	↑	–	–	–	–	↓	–	–	–
GPX	–	↓	↑	–	–	–	↑	–	–	–
GR	–	↓	↑	–	–	↓	↑	↓	↑	↓
Complex I	↑	–	–	–	–	–	–	–	–	–
Complex II	↑	–	–	–	–	–	–	–	–	–

Table 1, we summarized main neurochemical findings in mice following SIS and/or PA paradigms in voluntary wheel running.

Acknowledgments

This work was supported by CNPq (National Council for Research and Development, #403120/2012–8, #462333/2014-0, #306204/2014-2, #310113/2017–2, #150082/2018–5); ALD, AL, AFB and ALSR are recipients of CNPq Research Productivity Fellowship; FINEP Research Grant "Rede Instituto Brasileiro de Neurociência" (IBN-Net #01.06.0842-00); INCT Excitotoxicity and Neuroprotection; FAPESC (Foundation for the Support of Scientific and Technological Research in the State of Santa Catarina); MPC received a post-doctoral PNPD scholarships from CAPES (Coordination for the Improvement of Higher Education Personnel, Brazil).

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