RESEARCH PAPER

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Weekend warrior exercise model for protection from chronic mild stress-induced depression and ongoing cognitive impairment

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We aim to investigate the role and biological mechanisms of the weekend warrior (WW) exercise model on depression-induced rats in comparison to the continuous exercise (CE) model. Sedentary, WW, and CE rats were subjected to chronic mild stress (CMS) procedure. CMS and exercise protocols continued for six weeks. Anhedonia was evaluated by sucrose preference, depressive behavior by Porsolt, cognitive functions by object recognition and passive avoidance, and anxiety levels by open field and elevated plus maze. After behavioral assessments, brain tissue myeloperoxidase (MPO) activity, malondialdehyde (MDA) levels, superoxide dismutase and catalase activities and GSH content, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1β, cortisol and brain-derived neurotrophic factor levels and histological damage was assessed. CMS-induced depression-like outcomes with increases in anhedonia and decreases in cognitive measures that are rescued with both exercise models. The increased immobilization time in the Porsolt test was decreased with only WW. Exercise also normalized the suppression of antioxidant capacity and MPO increase induced by CMS in both exercise models. MDA levels also declined with both exercise models. Anxiety-like behavior, cortisol levels, and histological damage scores were exacerbated with depression and improved by both exercise models. TNF-α levels were depleted with both exercise models, and IL-6 only with WW. WW was as protective as CE in CMS-induced depression-like cognitive and behavioral changes *via* suppressing inflammatory processes and improving antioxidant capacity.

Key words: weekend warrior, exercise, depression, cognitive function, continuous exercise

INTRODUCTION

Depression is a common mental disorder characterized by depressed mood and/or anhedonia (loss of interest or pleasure) and related to poor concentration and cognitive impairment such as deficiency in executive functions, attention, psychomotor speed, and memory (Moussavi et al., 2007; Hammar and Ardal, 2009; Tolentino and Schmidt, 2018). On the other hand, depression is seen in a higher frequency among individuals with anxiety disorders, and increased anxiety levels are observed among depressive patients (Fava et al., 2000). Although the pathophysiology of depression is poorly understood, psychological and biological factors are suspected to be involved (England and Sim, 2009).

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A substantial body of evidence confirms the beneficial effects of regular physical activity on physiological and psychological fitness and verifies physical inactivity as a leading risk factor for mortality. Physical activity improves cardio-metabolic fitness, psychological health, and cognitive functions and reduces the risk of cardiovascular, metabolic, neurologic, and psychological diseases (Kasımay et al., 2006; 2010; Cakır et al., 2010; Conti et al., 2015; Ozbeyli et al., 2017; Koyuncuoğlu et al., 2021). Moderate-intensity continuous physical activity is a classical exercise model (also known as continuous exercise; CE) recommended to optimize the benefits of physical activity (ACSM's Guidelines for Exercise Testing and Prescription 2017). In this model, regular exercise is accumulated across the week for at least 150 min/week. However, compared to inactive individuals, participants may gain health benefits by through physical activity below the 150 min/week threshold. For example, exercising vigorously once or twice for at least 75 min/week or exercising moderately 2 days of the week for at least 150 min/week (known as "Weekend Warrior" (WW) physical activity pattern) is associated with improved endurance and performance and enhanced outcomes for Metabolic Syndrome and Diabetes Mellitus models (Alaca et al., 2018; Xiao et al., 2018).

For the aforementioned protective effects, CE is well-known to improve antioxidant capacity and enhance neuroplasticity by increasing neurotrophic and other growth factors. Moreover, CE induces neurogenesis and increases the volume of the hippocampus, prefrontal, and temporal regions that are essential for memory functions (Erickson et al., 2011). We previously reported that CE alleviates oxidative stress by suppressing inflammatory processes and by increasing antioxidant capacity in the brain to support positive effects on cognitive functions and anxiety (Ozbeyli et al., 2015; 2017, Koyuncuoğlu et al., 2021). Additionally, CE was shown to be associated with a low incidence of depression (Schuch et al., 2018). In contrast, one or two sessions of strenuous physical activity (the WW exercise model) can lower the risk of depression, but these beneficial effects are not consistently reported and the underlying mechanisms are unknown (Bernaards et al., 2006).

There is only one previous study that examined the role of WW on depression with no data concerning its possible impacts on cognitive functions (Hamer et al., 2017). Moreover, the protective effects of CE on depression-related cognitive dysfunction is not clear and need to be explored. Therefore, we investigated and compared the putative protective effects of WW and CE on depression-like behavioral deficits, and the contribution of neuroinflammation and oxidative stress as potential biological mechanisms.

METHODS

Animals

Male Sprague-Dawley rats (200-250 g, 6-8 weeks old, n=36) supplied by the Marmara University (MU) Animal Center (DEHAMER), were housed in relative humidity (65-70%) and temperature-controlled room (22±2°C) with standardized light/dark (12 h/12 h) cycles. The rats were fed with standard rat pellets and had free access to water. All experimental protocols were performed in accordance with the protocols approved by the MU Animal Care and Use Committee (approval no: 106.2018.mar), based on the Public Health Service Policy on Human Care and Use of Laboratory Animals.

Experimental design

Male rats (n=36) were randomly assigned to six groups according to exercise regime (Sedentary; SED, weekend warrior; WW, and continuous exercise; CE) and stress (chronic mild stress; CMS and controls). The experimental design is shown in Fig. 1. Exercise groups were habituated to the swimming pool (see below) on week -1. Both exercise protocols and CMS started on week 0 and continued for six weeks. All of the exercise groups performed swimming exercises for 150 min per week, for 6 weeks. CE groups were exercised 5 days per week, 30 min per day, and WW groups were exercised 2 days per week, 75 min per day (a total of 150 min/week). Body weight was recorded weekly and the sucrose preference test was performed fortnightly. At the beginning of the experiments, the elevated plus maze test was performed to measure baseline anxiety. In the last week of testing, a behavioral testing battery consisting of the object recognition test, passive avoidance, the Porsolt test, elevated plus maze and open field was administered with one test per day to reduce stress. After behavioral testing, the rats were decapitated and their brains were removed and washed with cold saline. Six samples were acquired per brain by first dissecting the brain sagittally, and then coronally twice for each half. The samples were frozen at -80°C to evaluate myeloperoxidase (MPO), superoxide dismutase (SOD) and catalase (CAT) activities, malondialdehyde (MDA) and glutathione (GSH), cortisol, tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, and brain-derived neurotrophic factor (BDNF) levels. Further gross histological analysis was also carried out as described below. In all groups, the same section of the brain was used for analysis.

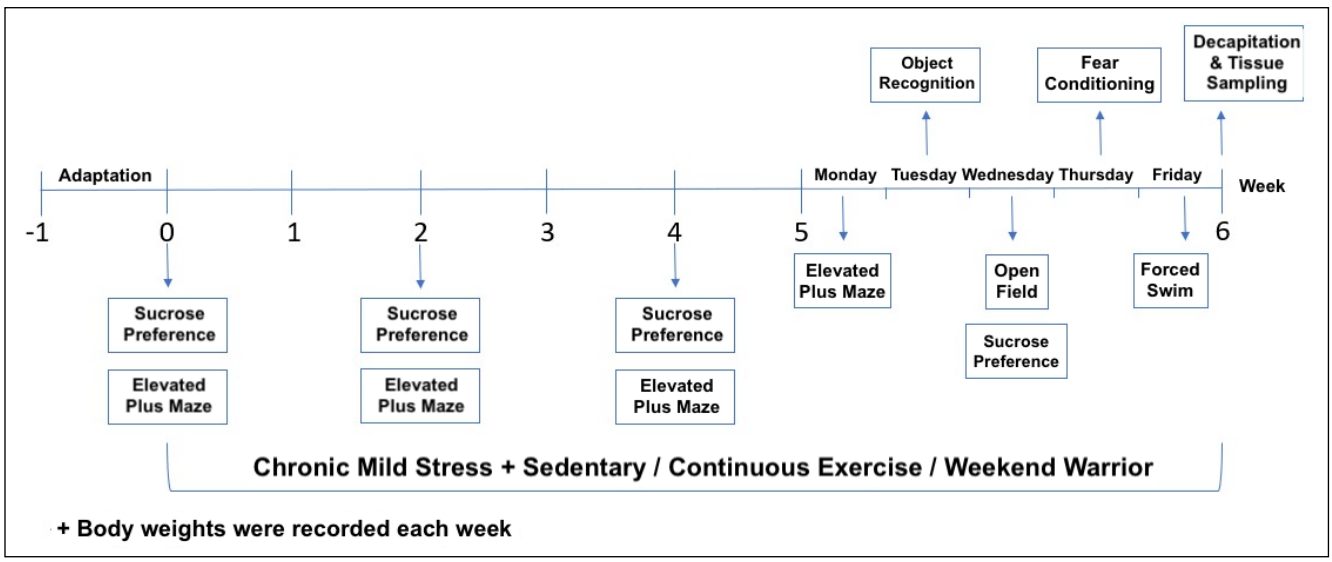

Fig. 1. Experimental design of non-stressed or CMS-induced groups, which were either sedentary or trained for 6 weeks according to continuous exercise or weekend warrior patterns. CMS; chronic mild stress.

Exercise protocols

The swimming training was done in a cylindrical plastic pool (diameter: 150 cm) with a 60 cm height filled with lukewarm water (31±1°C) to a depth of 45 cm. Rats were prevented from inactivity with gentle physical prompting with a stick. After the exercise was completed, the rats were dried and body temperature was maintained at 37°C using a hair dryer in warm mood (Ozbeyli et al., 2015).

Continuous exercise model: The rats were subjected to a moderate-intensity swimming exercise procedure: on the same 5 consecutive days of the week, for 6 weeks. The daily 30 min training sessions were conducted between 12:00 p.m. and 1:00 p.m. (Alaca et al., 2018).

Weekend warrior exercise model: The rats were subjected to a weekend warrior swimming procedure: on the same 2 consecutive days of the week, for 6 weeks. The training sessions lasting 75 min per day were conducted between 12:00 p.m. and 01:15 p.m. (Alaca et al., 2018).

Chronic mild stress procedure: As a model for depression, the CMS procedure was applied for 6 weeks. CMS is a rodent model where animals are exposed to a fixed sequence of mild stressors. Rats were subjected to nine different stressors. Our implementation of the model is described in Table 1 (Willner et al., 1987).

Sucrose preference test

The body weights were recorded every week on Mondays. The sucrose preference test to measure anhedonia. It is well-known that in an unstressed state, rats prefer sucrose solution over tap water. Stress exposure reduces the consumption and preference for sucrose. Low (1 to 2%) sucrose solution with tap water was shown to be effective (Huang et al., 2017).

Rats were placed in individual cages similar to their home cages in the holding room during the dark cycle. In the adaptation period, a bottle of 100 mL 1% (w/v) sucrose solution was placed in each cage for 24 h. Then, bottles were removed and the rats were deprived of food and water for 18 h. Afterward, 2 bottles were placed one containing 100 mL of sucrose solution (1%, w/v) and the other, 100 mL of tap water, in each cage for 4 h. The volumes of consumption for

Table 1. Chronic mild stress protocol.

Day	Stressors
Monday	10.00 a.m.: Soiled cage (with 200 mL of water spilled in the bedding) for 24 h
Tuesday	10.00 a.m.: Tilted cage (45° along the vertical axis for 24 h) 03.00 p.m.: Food and water deprivation (3 h)
Wednesday	03.00 p.m.: Cage shaking (10 min)
Thursday	10.00 a.m.: Partner exchange (24 h) 03.00 p.m.: Tail squeezing (1 min)
Friday	10.00 a.m.: No bedding (3 h) 03.00 p.m.: Exposure to empty water bottle (3 h)

both solutions were recorded and sucrose preference was calculated by:

Sucrose preference index (%): 100 × sucrose solution intake (mL) / total amount of liquid intake (mL) (Krishnan et al., 2007).

Decreased sucrose preference index indicates anhedonia, which is a major symptom of clinical depression.

Porsolt test

The Porsolt test is based on the observation that rats would increase time spent immobile in inescapable swimming stress as a sign of behavioral despair (Porsolt et al., 1978). The rats were placed in a transparent plexiglass tank (50 cm height and 18 cm diameter) filled with water at 35 cm. The water temperature was kept constant (24±1°C). We first exposed rats to a 15 min session to facilitate the latency to immobility on the test day 24 h later. The increased immobilization time on the test day indicates increased despair in the rat.

Evaluation of cognitive functions

Object recognition test: The object recognition test was performed to evaluate working memory. A white melamine-coated open box with dimensions of 50 cm × 50 cm × 50 cm was used for the test. The test has three phases: acclimatization, familiarization with the sample objects, and the introduction of a novel object. Rats were allowed to explore the apparatus for 10 min during acclimatization. A day after, 2 identical sample objects were placed in the box, and rats are placed into the apparatus facing away from the objects. A 10 min session was recorded by a video camera and rats were returned to their homecages after the session. Sixty min after, one of the sample objects was replaced with a novel object and a 3 min session was recorded with rats re-introduced to the apparatus, facing away from the objects. The videos were analyzed by two experimenters. Contact with the object was defined as physical contact with the animals' noses to an object. Object recognition was evaluated by taking the difference between the time spent with the sample and the novel objects according to the following formula (Bevins and Besheer, 2006):

Difference score (centisecond *): time spent with the novel object - time spent with the sample object.

* 1 second = 100 centiseconds

An increase in difference scores was evaluated as an improvement in object recognition. The apparatus and all objects were cleaned by using 90% isopropyl alcohol and a floor sheet was changed in each trial.

Passive avoidance test: Fear conditioning is a behavioral paradigm as a model of aversion learning (Buccafusco, 2009). Passive avoidance test was used for analyzing fear conditioning. A specific apparatus that included a light and a dark compartment (30 cm × 30 cm × 40 cm) was used. The compartments were identical, composed of white melamine-coated wood, and separated by a manually controlled guillotine door (8 cm × 12 cm). To minimize light leaking into the dark compartment, the whole dark compartment was covered with blackout film. The floors of both compartments were covered by thick plastic transparent films to be cleaned easily and to reduce the odor clues left by the previous rat. A small hole was positioned on the wall of the dark compartment opposite the guillotine door (3 cm from the floor) for applying air puffs from an air-duster canister (Electrolube, GDP, UK).

The protocol is based on Moriarty (2012). For the habituation period, the rat was placed in the light compartment facing away from the guillotine door and was allowed to explore both of the compartments for 5 min before being returned to its cage. On the following day, the rat was placed in the light compartment in the same position and the time taken to transition into the dark compartment was noted. When the rat entered the dark compartment, the guillotine door was closed manually by the experimenter. A single, brief air puff (Air-Duster Plus, Electrolube, duration of 1 s) was applied when the rat's head is near the hole in the wall. After the air puff rats were kept in the dark compartment for 90 s before being returned to their homecages. Rats that did not pass into the dark compartment in less than 300 s were excluded from the study. One hour after the acquisition trial, a retention trial was applied for evaluating passive avoidance. The rat was located in the same position in the light compartment, facing away from the door and the latency to cross over to the dark compartment was recorded. If the rat did not enter into the dark compartment, the latency period was noted as 300 s. During the retention trial, no air puff was applied. The rats were expected to delay their entry to the dark compartment due to the presumed aversion to the previously experienced air puff. The increasing latency indicated an improvement in short-term aversive memory. In all trials, the compartments were cleaned before and between sessions with 90% isopropyl alcohol (Moriarty et al., 2012).

Anxiety-like behavior measurements

Open field test: The open field test (OFT) was performed to measure anxiety-like behavior. Briefly, a wooden apparatus consisting of 4 walls (with a height

of 40 cm) and a square bottom (100 cm × 100 cm) was used. The bottom of the apparatus was divided into 16 (20 cm × 20 cm) equal squares. A camera was installed above the OFT apparatus. For each session, a single rat was placed in the center of the arena. In a quiet room with dim lighting, four different behavior pattern was recorded for 5 min: the number of squares crossed (with the four paws), the number of rears (standing on rear limbs with or without touching the walls), time spent in the center (duration of time spent in the middle 4 squares), and freezing time (time spent motionless). After the trial, the rat was returned to its home cage. Then, the arena was cleaned with a 90% isopropyl alcohol solution (Prut and Belzung, 2003). The 5-min video records were scored by two experimenters. The decrease in the number of squares crossed, a decline in the number of rears, a decrease in time spent in the center, and prolonged freezing time are indicative of increased anxiety.

Elevated plus maze test: The elevated plus maze has 2 enclosed arms (50 cm ×10 cm) opposed perpendicularly by 2 open arms (50 cm ×10 cm) with an open roof and is raised 60 cm above the floor. The rats were placed in the center of the apparatus facing one of the open arms and the session is recorded with a video camera for 5 min. The entry into the arm was defined as the entry of the head and at least half of the body. Time spent in open and closed arms was evaluated by 2 experimenters. The percentage of time spent in the open arms was calculated by the following formula:

Percentage of time spent in the open arms (%) = 100 × time spent in open arms / (time spent in open arms + time spent in closed arms).

Decreased percentage of time spent in the open arms is an indicator of higher levels of anxiety (Pellow et al., 1985).

Oxidative stress parameters and antioxidant capacity markers

Measurement of MPO activity: For analysis of tissue MPO activity as an indicator of neutrophil infiltration, brain tissue samples (250-300 mg) were diluted in 10 volumes of 0.5% HETAB (in 50 mM potassium phosphate buffer; pH:6) and homogenized. After this step, it was centrifuged at 12,000 rpm for 10 min at 4°C. After removing the supernatant, the pellet was re-homogenized with an equivalent volume of 50 mM K_2HPO_4 containing 0.5% HETAB and 10 mM EDTA. MPO activity was assessed by measuring the H_2O_2-dependent oxidation of o-dianisidine · 2HCl. One unit of enzyme activity was defined as the amount of MPO present per gram of tissue weight that caused a change in absorbance of 1.0 min^{-1} at 460 nm and 37°C. MPO activity was expressed as units per gram tissue (U/g tissue) (Bradley et al., 1982).

Measurement of MDA and GSH levels: As an end product of oxidative stress and lipid peroxidation, MDA and GSH were measured. Brain tissue samples (150-300 mg) were homogenized with a tissue homogenizer (Ultra Turrax) by using a 10% trichloroacetic acid (TCA) solution and then centrifuged at 3,000 rpm for 15 min at 4°C. The supernatant was removed and the sample was recentrifuged at 15,000 rpm at 4°C for 8 min. Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and results were expressed as nanomoles MDA per gram tissue (nmol/g) (Buege and Aust, 1978).

GSH measurements were performed by a spectrophotometric method using a modification of the Ellman (Aykaç et al., 1985). Briefly, after centrifugation at 3,000 rpm for 10 min, 0.5 mL of supernatant was added to 2 mL of 0.3 mol/L $Na_2HPO_4 \cdot 2H_2O$ solution. A 0.2 mL solution of dithiobisnitrobenzoate (0.4 mg/mL 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of 1.36×10^4 M^{-1} cm^{-1} and results were expressed in micromoles of GSH per gram tissue (μmol/g).

Measurement of SOD and CAT activity: SOD and CAT activities indicate the antioxidant capacity of the tissue (Zhang et al., 2017). SOD is a specific antioxidant enzyme that catalyzes the dismutation of oxygen and hydrogen peroxide. SOD activity is measured by its ability to increase the photooxidation rate of o-dianisidin sensitized by riboflavin. The superoxide radical formed by the riboflavin exposed to fluorescence light; by the action of the SOD in the media, is converted into hydrogen peroxide. Then hydrogen peroxide reacts with the o-dianisidin to form a colored product. The absorbance of the product is evaluated spectrophotometrically at 460 nm. With the help of the standard graphic and considering the dilutions, the SOD activity of the supernatant was calculated in the form of units per milligram protein (U/mg protein) (Mylroie et al., 1986).

CAT directs the reaction by which hydrogen peroxide is decomposed to water and oxygen. This conversion was observed as a decrease in absorbance at 240 nm. Catalase measurements were performed as described previously and expressed as units per milligram protein (U/mg protein) (Aebi, 1984).

Measurement of brain tissue cortisol, TNF-α, IL-1β, IL-6, and BDNF levels: The concentrations of TNF-α, IL-1β, IL-6, BDNF, and cortisol levels were measured in rat brain tissues by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Bioassay Tech., Shanghai, China). The brain tissues

were homogenized with zirconium magnetic beads (Next Advance-Bullet Blender) and assays were performed according to the manufacturer's instructions. The wavelength of the microplate reader (EnSpire – PerkinElmer) was 450 nm. The data were determined using a standard curve prepared for each assay and expressed as picograms or nanograms of cytokine/chemokine/cortisol per g of brain tissue (Sheikhzadeh et al., 2015).

Histological analysis

For light microscopic evaluation, the brain tissues of animals were fixed in a 10% neutral buffered formalin. Tissue samples were dehydrated in graded ethanol series (70, 90, 96, and 100%), cleared in toluene and embedded in paraffin. Paraffin sections (5-µm) were cut by a rotary microtome and mounted on slides. Sections were then deparaffinized and stained with hematoxylin and eosin to visualize the general cytoarchitecture and the severity of neuronal damage in the cortex and dentate gyrus (DG) and CA3 regions of the hippocampus. Sections were examined under a light microscope (Olympus BX51, Tokyo, Japan) and photographs were taken. The severity of neuronal damage was scored semiquantitatively as follows: 0 = no damage, 1 = mild damage, 2 = moderate damage, and 3 = severe damage. The average damage scores were calculated for each group (Koyuncuoğlu et al., 2021).

Data analysis

GraphPad Prism statistical software (version 6.0) was used for evaluating the data. All data were expressed as mean ± SEM. For the comparison between multiple groups, Tukey- Kramer tests were used following one-way ANOVA. To compare between two groups, Student t-tests were used. The p-value was accepted to be statistically significant when it was less than 0.05 (P<0.05).

RESULTS

Body weight measurements

Throughout weight follow-up, all rats except for the CMS+SED group showed a significant increase in body weight compared to the previous week (P<0.001), whereas no significant difference was observed in weekly measurements between groups (First week $F_{(5,30)}$=1.351, P>0.05; 2nd week $F_{(5,30)}$=1.854, P>0.05; 3rd week $F_{(5,30)}$=0.640, P>0.05; 4th week $F_{(5,30)}$=0.634, P>0.05; 5th week $F_{(5,30)}$=0.868, P>0.05; 6th week $F_{(5,30)}$=0.636, P>0.05). No additional weight increase was observed between the 6th and 5th week measurements in the CMS+SED group, showing a cessation in weight gain with depression induction only in the SED group in the last week (Fig. 2).

Sucrose preference and Porsolt tests

In the sucrose preference test for anhedonia, there was no significant difference between the groups at weeks 0, 2, and 4 (data not shown), while week 6 results showed that the sucrose preference index decreased in the CMS+SED group compared to the Non-stressed SED group (P<0.05), and both exercise applications significantly reversed this decrease (P<0.05). Among the CMS groups, this return was also significantly higher in the WW group when compared to the CE group (P<0.05) (Fig. 3A).

Freezing time was increased with CMS in the SED group (P<0.05) and reduced in the WW group (P<0.05). $F_{(5,27)}$ =3.313, P< 0.05 (Fig. 3B).

Cognitive function tests

The difference score (time difference spent with the novel and sample objects) was decreased significantly in the CMS+SED group when compared with the non-stressed SED group (P<0.05). The same scores were increased with both of the exercise patterns+CMS (P<0.05-0.001). The scores were also increased with both of the exercise patterns in non-stressed groups (P<0.05-0.01) $F_{(5,30)}$=4.121, P<0.01 (Fig. 4A). The passive avoidance latency was shorter in CMS+SED group com-

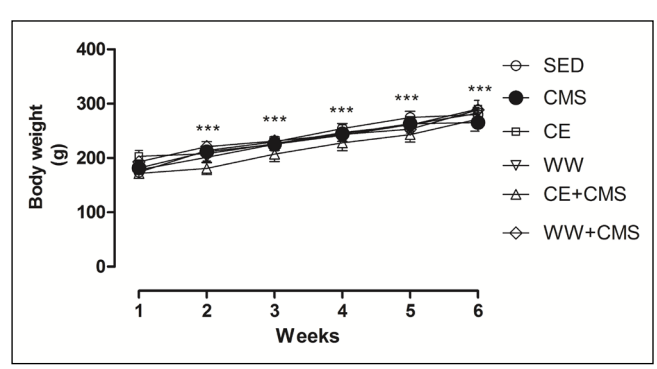

Fig. 2. Body weight measurements of non-stressed or CMS-induced rats, which were either sedentary or trained for 6 weeks according to CE or WW patterns. SED; sedentary, CE; continuous exercise, WW; weekend warrior, CMS; chronic mild stress. ***p<0.001, compared to previous week (n=6/group).

pared with the non-stressed SED group (P<0.05), and normalized by CE (P<0.05) $F_{(5,29)}$=3.314, P<0.05 (Fig. 4B).

Anxiety-like behavior measurements

CMS+SED decreased the number of square crossing, rearing, and time spent in the center in the open field (P<0.05) while immobility was increased in the CMS+SED group compared to non-stressed SED group (P<0.05) (Fig. 5A-D). These parameters were normalized by CE (P<0.05) and the time spent in the center was also normalized to the control levels in CMS+WW (P<0.05). In non-stressed groups, there were increases in square crossings and rearing in both of the exercise models (P<0.05-0.001). Time spent in the center and immobility was improved by WW (P<0.05). Number of square crossings $F_{(5,28)}$=9.627, P<0.0001; time spent in the center $F_{(5,29)}$=7.353, P<0.001; rears $F_{(5,30)}$=12.32, P<0.0001; immobility duration $F_{(5,30)}$=5.538, P<0.01 (Fig. 5A-D).

In the elevated plus maze test, time spent in open arms was decreased in the CMS+SED group at the end of week 6 compared to week 0 (P<0.05), whereas no decrease was observed in the CMS groups treated with exercise. $F_{(5,30)}$=1.659, P>0.05 (Fig. 5E).

Oxidative stress parameters and antioxidant capacity markers

MPO activity, an indicator of neutrophil infiltration through the tissue, was increased in the CMS+SED group (P<0.05) and significantly lowered in both exercise groups (P<0.05) $F_{(5,30)}$=3.31, P<0.05 (Fig. 6A).

As a marker of lipid peroxidation, the MDA levels were decreased with both exercise protocols not only in depression-induced conditions but also in non-stressed conditions compared to the corresponding SED group (P<0.05-0.01). $F_{(5,24)}$=7.855, P<0.001 (Fig. 6B).

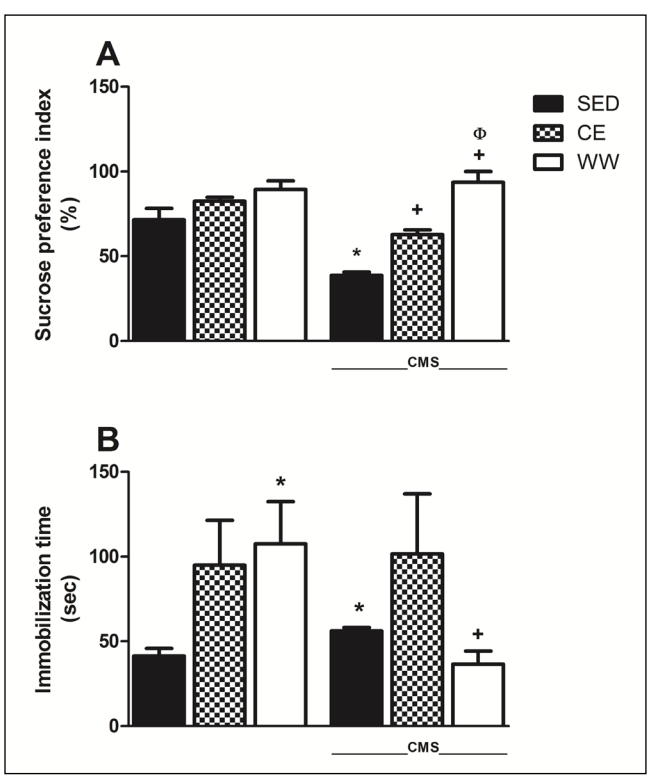

Fig. 3. Sucrose preference and Porsolt test results of non-stressed or CMS-induced rats, which were either sedentary or trained for 6 weeks according to CE or WW patterns. Sucrose preference index (A), immobilization time (B). Sucrose preference index (%): 100 × Sucrose solution intake (mL) / Total amount of liquid intake (mL). SED; sedentary, CE; continuous exercise, WW; weekend warrior, CMS; chronic mild stress, sec; second. *p<0.05, compared to non-stressed SED group. +p<0.05, compared to CMS-induced SED group, p<0.05, compared to corresponding CE group (n=6/group).

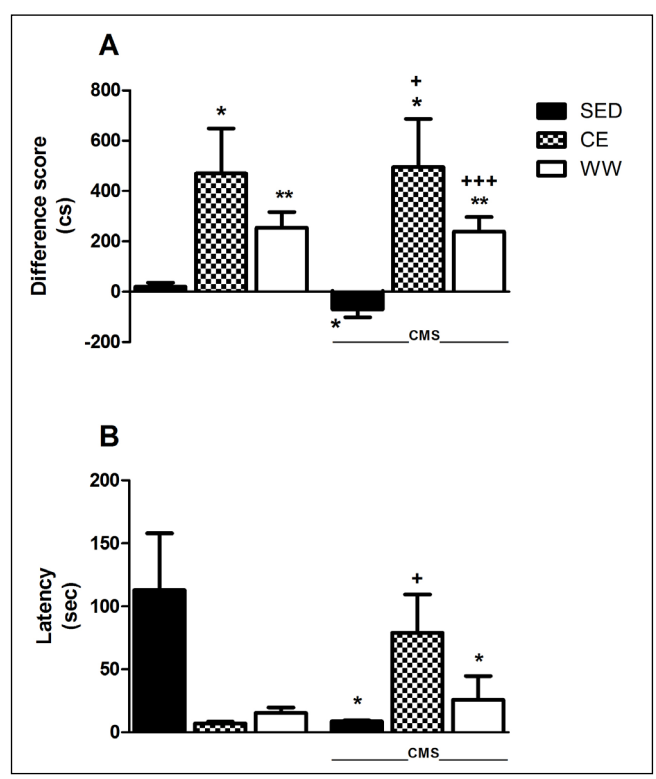

Fig. 4. Novel object recognition and fear conditioning test results of non-stressed or CMS-induced groups, which were either sedentary or trained for 6 weeks according to CE or WW patterns. Difference score (A), latency (B). Difference score (cs): Time spent with the novel object – time spent with the sample object. Latency (sec): latency to enter the dark compartment. SED; sedentary, CE; continuous exercise, WW; weekend warrior, cs; centisecond, CMS; chronic mild stress, sec; second. *p<0.05, **p<0.01, compared to Non-stressed SED group; +p<0.05, +++p<0.001, compared to CMS-induced SED group (n=6/group).

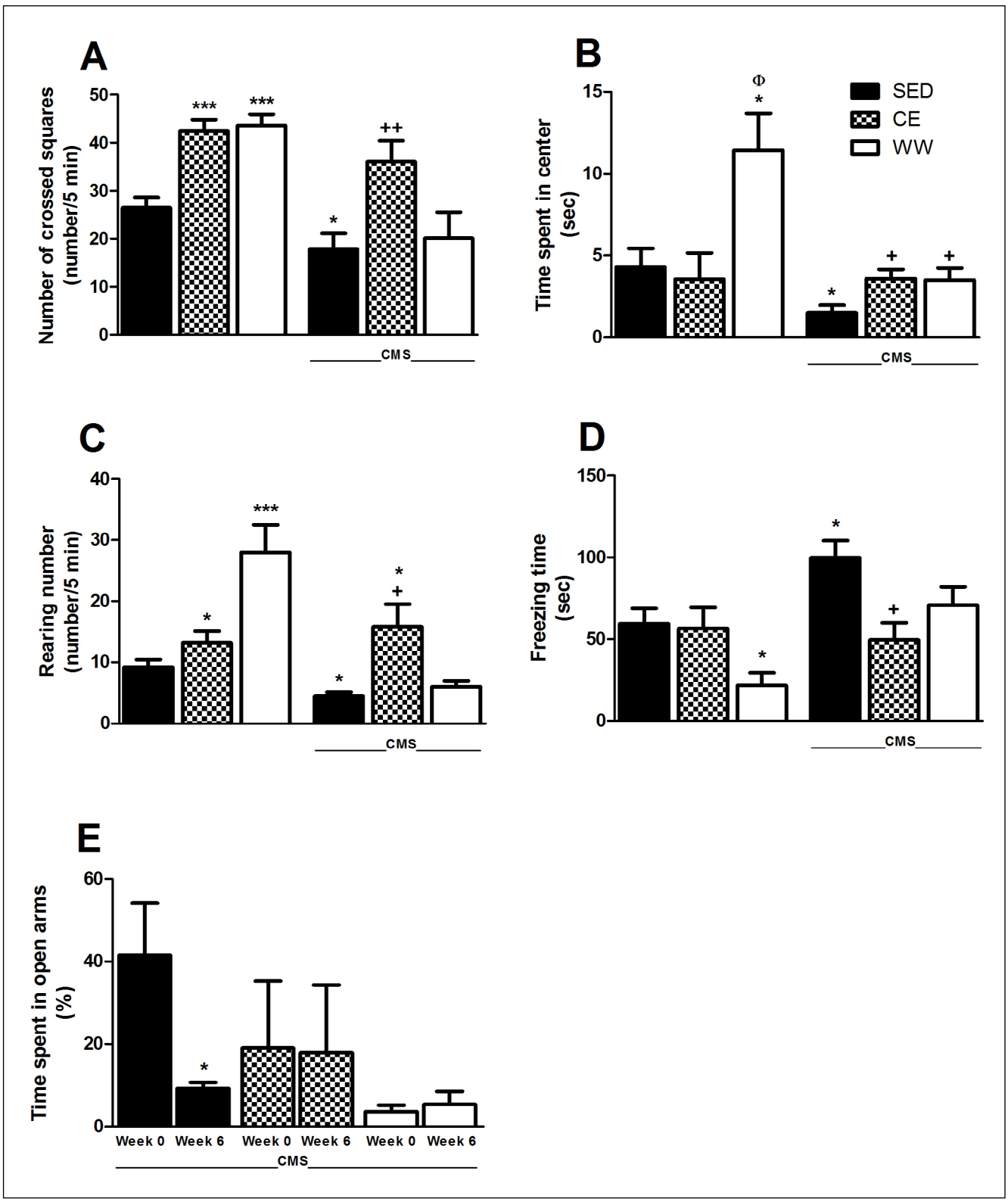

Fig. 5. Open field and elevated plus maze test results of non-stressed or CMS-induced groups, which were either sedentary or trained for 6 weeks according to CE or WW patterns. Rearing number (A), number of crossed squares (B), time spent in center (C), freezing time (D), time spent in open arms (E). Percentage of time spent in the open arms (%)=100 x time spent in open arms / (time spent in open arms + time spent in closed arms). SED; sedentary, CE; continuous exercise, WW; weekend warrior, CMS; chronic mild stress, sec; second. *p<0.05, ***p<0.001, compared to non-stressed SED group; +p<0.05, compared to CMS-induced SED group, p<0.05, compared to corresponding CE group (n=6/group).

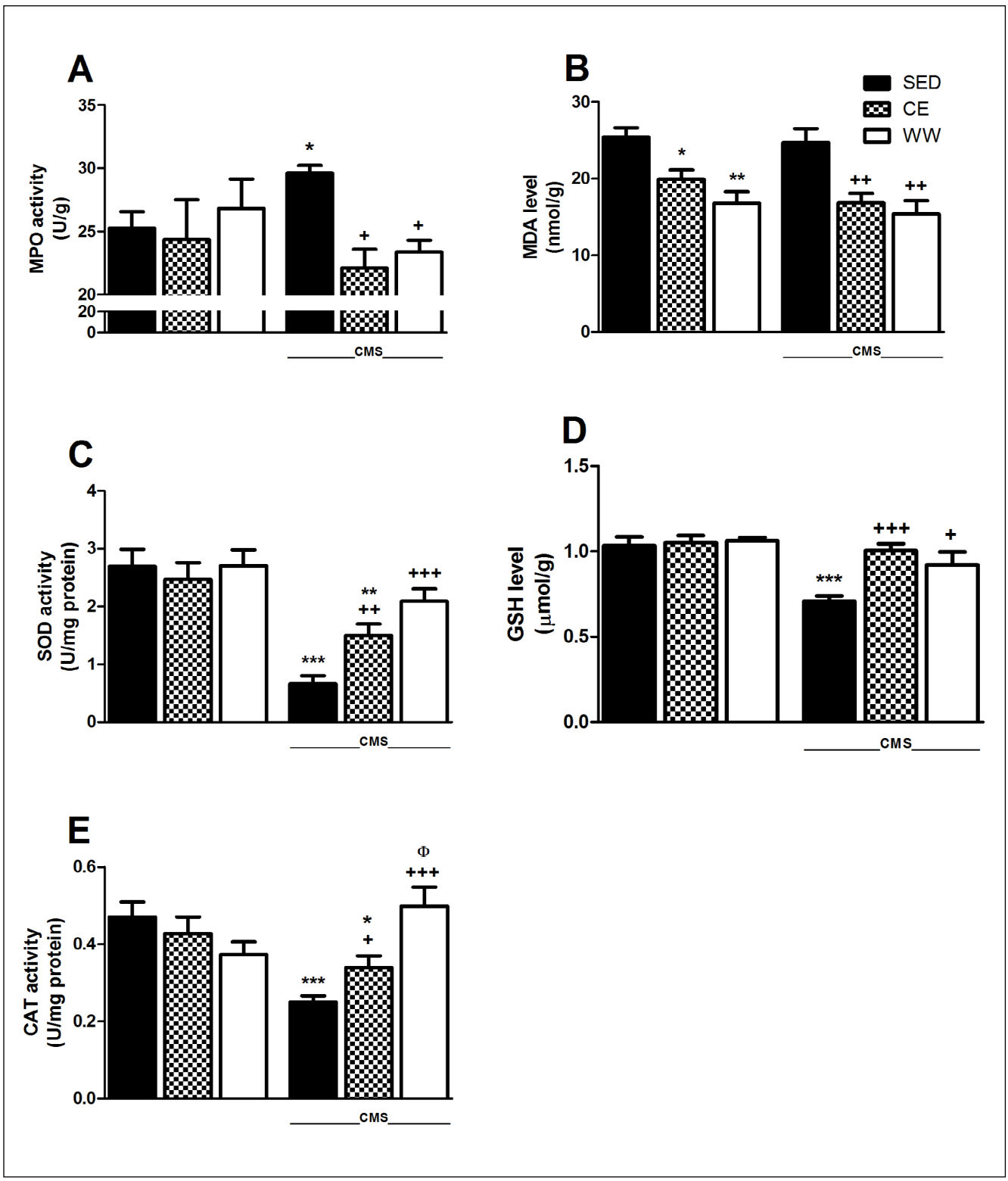

Fig. 6. Oxidative stress parameters and antioxidant capacity markers in the brain tissue of non-stressed or CMS-induced groups, which were either sedentary or trained for 6 weeks according to CE or WW patterns. Myeloperoxidase (MPO) (A) activity, malondialdehyde (MDA) (B) and glutathione (GSH) levels (C), catalase (CAT) (D) and superoxide dismutase (SOD) activity (E). SED; sedentary, CE; continuous exercise, WW; weekend warrior, CMS; chronic mild stress. *p<0.05, **p<0.01, ***p<0.001, compared to non-stressed SED group; +p<0.05, ++p<0.01, +++p<0.001, compared to CMS-induced SED group, p<0.05, compared to corresponding CE group (n=6/group). U/g; Unites per gram tissue, nmol/g nanomoles per gram tissue, µmol/g; micromoles per gram tissue; U/mg protein; Unites per milligram protein.

Although antioxidant GSH levels, SOD, and CAT activities were all reduced in the CMS+SED group (P<0.001), they were all improved with both exercise protocols (P<0.05-0.001). SOD $F_{(5,30)}$=11.03, P<0.0001; GSH $F_{(5,29)}$=8.571, P<0.0001; CAT $F_{(5,30)}$=6.291, P<0.001 (Fig. 6C-E). Moreover, among the CMS groups, WW increased CAT activity levels more than CE (P<0.05), underlying its role in restoring antioxidant capacity.

Cortisol, TNF-α, IL-1β, IL-6 and BDNF measurements

As expected, the cortisol levels increased in the CMS+SED group (P<0.05) and declined significantly with both exercise protocols (P<0.05-0.001). $F_{(5,27)}$=3.324, P<0.05 (Table 2).

Although TNF-α levels were not changed significantly in the CMS+SED group, their levels were suppressed by WW in CMS or non-stressed groups compared to the corresponding SED groups (P<0.05-0.01). Additionally, the levels were also diminished with CE among the CMS groups (P<0.05). $F_{(5,28)}$=3.314, P<0.05 (Table 2). As another pro-inflammatory cytokine, IL-6 levels also decreased significantly in the WW+CMS group compared to the SED group (P<0.05). $F_{(5,30)}$=0.954, P>0.05 (Table 2). There was no significant difference in IL-1-β and BDNF levels between the groups. IL-1β $F_{(5,28)}$=0.317, P>0.05; BDNF $F_{(5,29)}$=1.362, *P>0.05*.

Histological evaluation

Microscopic analysis

Histological analysis revealed regular neurons with large nuclei and distinct nucleoli in the cortices and hippocampus of rats in the non-stressed SED, CE, and WW groups (Fig. 7). Compared with the SED group, neuronal damage, pyknotic cell nuclei, cytoplasmic deterioration, and irregularity of cell structures were detected in the CMS+SED group. Among the CMS groups, neuronal degeneration was decreased in the CE and WW groups compared to the SED group in the cortex and hippocampus, mainly the CA3 region.

Microscopic damage scores

Brain sections were evaluated semi-quantitatively to yield microscopic score graphs of microscopic damage (Fig. 8A-C). Evaluations made in the cortex, CA3, and DG region of the hippocampus indicated the histological scores were increased in the CMS+ SED group compared with the non-stressed SED group (P<0.001), except in the DG region, the scores were decreased with both exercise models (P<0.05-0.001). CA3 $F_{(5,27)}$=8.221, P<0.0001; DG $F_{(5,27)}$=11,49, P<0.0001; cortex $F_{(5,30)}$=23.52, P<0.0001.

DISCUSSION

The present study was undertaken primarily to examine the possible protective effects of the WW exercise regime on CMS-induced cognitive impairment. In addition, we aimed to compare the outcomes of WW with CE, a more conventional exercise regime in order to develop an alternative preventative treatment for depression. Thirdly, we aimed to identify the underlying biological mechanisms. In addition to the beneficial effects of CE on depression, our results show a comparable benefit with WW in improving CMS-induced depression-like behaviors. Both of the exercise methods had ameliorating effects on the cortical and

Table 2. Cortisol, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1beta (IL-1β), brain derived neurotrophic factor (BDNF) assays in the brain tissue of non-stressed or CMS-induced rats, which were either sedentary or trained for 6 weeks according to CE or WW patterns. CMS; chronic mild stress, SED; sedentary, CE; continuous exercise, WW; weekend warrior.

	SED	CE	WW	CMS		
				SED	CE	WW
Cortisol (ng/g tissue)	286.8±12.16	284.6±48.01	265.2±28.52	321.6±8.34*	215.4±18.9+++	272.9±14.94+
TNF-α (µg/g tissue)	1.59±0.22	1.47±0.30	1.07±54.83*	1.36±84.24	1.12±0.06+	1.02±0.05++
IL-6 (ng/g tissue)	52.37±4.75	47.69±13.01	63.26±14.35	43.3±3.69	52.6±6.32	38.91±1.53*
IL-1β (ng/g tissue)	8.8±1.10	8.34±1.63	7.92±1.27	7.0±0.86	8.25±1.29	7.34±0.75
BDNF (ng/g tissue)	16.49±1.43	12.56±2.45	11.53±2.43	14.30±0.89	11.44±2.15	10.56±1.77

*p<0.05, compared to non-stressed sedentary group; +p<0.05, ++p<0.01, +++p<0.001, compared to CMS-induced sedentary group (n=6/group).

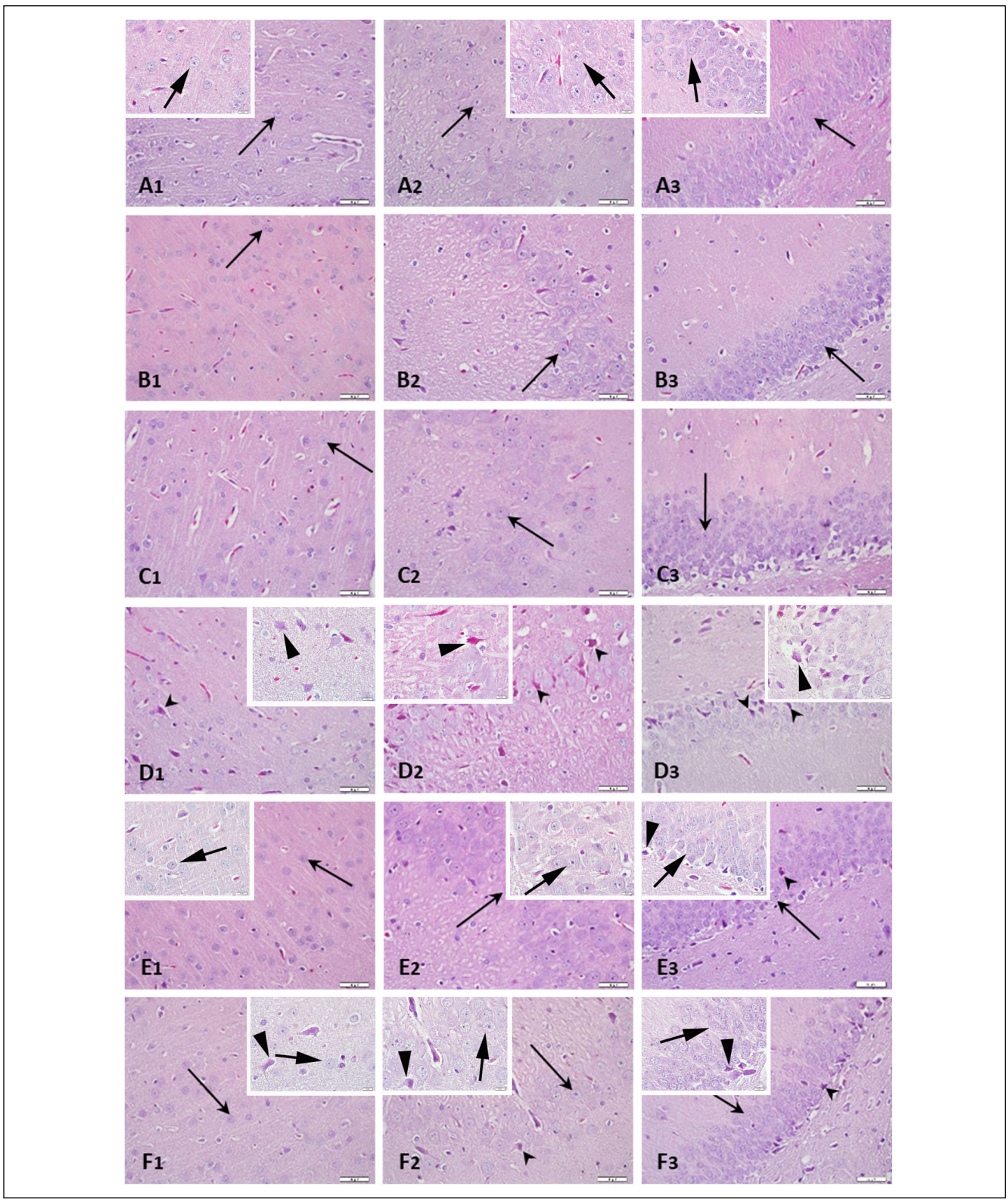

Fig. 7. Photomicrographs of cortex (A1-F1) and hippocampal CA3 (A2-F2) and DG (A3-F3) regions of rats from SED (A1-3), CE (B1-3), WW (C1-3), CMS-induced SED (D1-3), CMS-induced CE (E1-3), CMS-induced WW (F1-3) groups. Regular round neurons with large nuclei and prominent nucleoli are observed in the SED, CE and WW groups. Increased degeneration of neurons in CMS-induced SED group compared to controls is decreased in the CMS-induced CE/WW groups. SED; sedentary, CE; continuous exercise, WW; weekend warrior, CMS; chronic mild stress. Arrows: normal appearing neurons; arrowheads: degenerated neurons. Hematoxylin and eosin staining. Scale bars: 50 μm. Scale bars for insets: 10 μm.

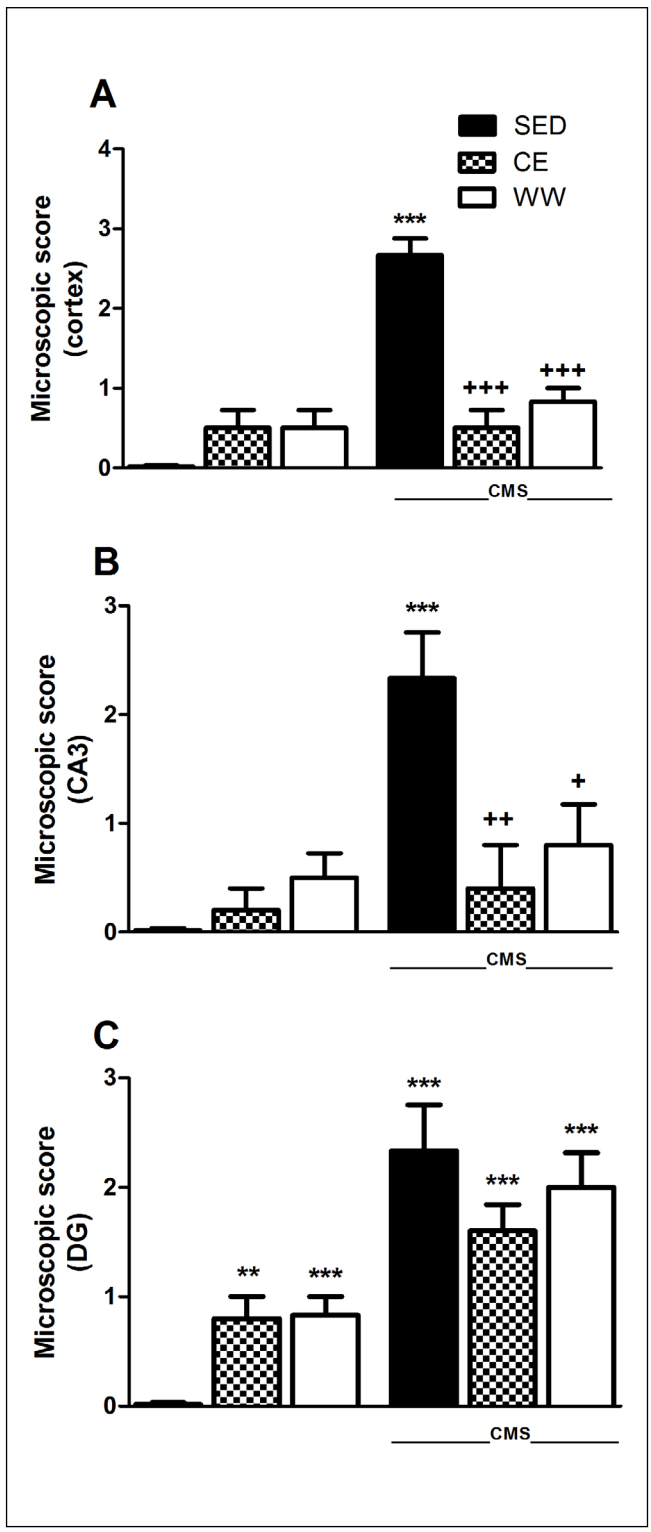

Fig. 8. Microscopic scores of the cortex (A), CA3 (B) and dentate gyrus (DG) (C) in the the brain tissue of non-stressed or CMS-induced rats, which were either sedentary or trained for 6 weeks according to CE or WW patterns. SED; sedentary, CE; continuous exercise, WW; weekend warrior, CMS; chronic mild stress. ***p<0.001, compared to non-stressed SED group; +p<0.05, ++p<0.01, +++p<0.001, compared to CMS-induced SED group (n=6/group).

hippocampal neuronal damage related to CMS, putatively by suppressing oxidative stress by modulating MPO and MDA levels, and *via* improving antioxidant capacity mediated by GSH, SOD, and CAT.

As depression is a prevalent mental disorder that is described by depressed mood and/or anhedonia, sucrose preference and Porsolt tests are commonly used in animal models of depression (Scheggi et al., 2018). In the present study, the sucrose preference index was significantly decreased and immobilization time was increased in the Porsolt test with CMS (Du et al., 2020). These results revealed that CMS was effective in modeling cardinal symptoms of clinical depression as the animals display anhedonia and depressive mood-like behavior in the 6th week (Willner et al., 1987).

Previously, Lee et al. (2015) reported reported that treadmill running as a form of CE had antidepressant effects in CMS-treated male Sprague-Dawley rats. In our study, decreased sucrose consumption levels were normalized in CMS groups with either form of exercise. In a recent study, CE improved not only sucrose preference but also immobility in the Porsolt test (Luo et al., 2019). In the present study, while CE was not found to be effective for decreasing immobility in the Porsolt test, WW was effective. WW was more effective in normalizing sucrose preference, and along with its effectiveness in reducing immobility in the Porsolt test, we propose it may be a better exercise regime in combating depression. Also, the sucrose preference index in the non-stressed groups was not significantly different, and the observed changes in the CMS groups were unlikely to be an increase in metabolic demand induced by exercise.

The lack of weight gain coincides with the development of depression-like deficits in CMS-treated animals in the 6th week. The role of stress on weight gain is consistent with the literature (Herrera-Pérez et al., 2016). Both CE and WW negated the effect of CMS on weight gain, adding to our convergent evidence on the beneficial effects of exercise in reversing depression-like symptoms.

Depression is known to have a negative impact on cognitive functions. The hippocampus has an important role in cognition, and its volume is reduced in depression (Suwabe et al., 2018). Consistent with findings in humans, CMS exposure affects short-term memory and other cognitive dysfunction (Henningsen et al., 2009). In the present study, CMS resulted in poorer novel object recognition and shorter latency to cross to the aversive compartment, suggesting cognitive deficits in short-term memory and in the learning of unpleasant experiences. Although the effect of different exercise models on CMS-induced cognitive deficit was unclear, our group previously reported dif-

ferent CE types to improve cognition in Alzheimer's disease, post-traumatic stress disorder, and acute stress-induced neuronal damage (Ozbeyli et al., 2015; 2017; Koyuncuoğlu et al., 2021). In the present study, we show for the first time, CMS-induced cognitive deficits and signs of neurodegenerations are ameliorated by WW as well as CE. Moreover, our results show exercise is beneficial in cognition in non-stressed conditions. Overall, our data link the therapeutic effects of CE and WW in cognition and neurodegeneration in their application for treating depression.

Stress blunts explorative behaviors and increases anxiety-like behaviors such as reduced locomotion and increased immobility, as well as escalating circulating cortisol (Roozendaal, 2002; Cox et al., 2011; Zhu et al., 2018; Luo et al., 2019). Our open field and elevated plus maze data are consistent with previous observations. In addition to increased defensive behavior, cortisol levels in the brain tissue were also increased. As an underlying mechanism to explain the effects of anxiety level on cognitive functions, the processes related to learning and memory formation are known to be affected with an inverted U-shaped function, such as low anxiety level improves learning, however high anxiety level inhibits it (Roozendaal, 2002). Therefore, aggravated anxiety levels with depression may have a role in the exacerbation of cognitive functions.

Physical activity has been suggested to improve cognition and mental health in general. According to the results of OFT, regular exercise was reported to have beneficial effects on increased anxiety levels of rats with depression (Luo et al., 2019). In another rat study, exercise is able to increase the amount of time spent on open arms in an elevated plus maze in CMS-treated animals (Lapmanee et al., 2013). We found CE increases the number of squares crossed and rearing non-stressed conditions and improved all OFT measures in CMS conditions. WW was effective in alleviating basal anxiety levels and prolonged time spent in the center and decreased freezing time following CMS. In sum, our behavioral data suggest both CE and WW are effective in reducing anxiety and depression-like behaviors (perhaps through regulating stress hormone action), WW appears to be more effective than CE in non-stressed animals while CE is more effective in CMS-treated animals.

Previous studies suggest that depression destabilizes the balance between the oxidative and antioxidative systems (Sarandol et al., 2007; Gałecki et al., 2009). In the present study, the antioxidants such as GSH, SOD, and CAT activities were found to be suppressed in the CMS brain. In olfactory bulbectomized rats (as an alternative model of rodent depression), CAT activity and GSH levels were decreased (Tasset et al., 2008). Likewise, in chronic unpredictable mild stress-induced rats that developed depression-like behaviors, GSH and serum total antioxidant capacity levels were depleted (Abd El-Fattah et al., 2018). Consequently, our data suggest that the reduction in antioxidant capacity may be a result of the change in oxidative load or the neurodegenerative processes accompanying CMS.

Regular exercise protects from the harmful effects of oxidative stress by improving antioxidant capacity. This upregulation is a result of an increase in free radicals stimulating the mitochondrial biosynthesis of antioxidant enzymes (Greathouse et al., 2005). In a previous study examining the effects of treadmill exercise on antioxidant capacity, the SOD, CAT, and glutathione peroxidase activity levels were found to be increased in the soleus muscle of exercising young rats (Lambertucci et al., 2007). In our previous studies, different types of CE such as swimming, rotarod exercise, walking on a treadmill, resistance training, and combined exercise models were reported to improve antioxidant SOD activity, GSH, and total sulfhydryl levels (Kasımay et al., 2006; 2010; Cakır et al., 2010; Kuru et al., 2015; Ozbeyli et al., 2017; Koyuncuoğlu et al., 2021). However, this is the first time we show that WW can improve antioxidant capacity through modulating GSH content, SOD, and CAT activity levels in the brain.

Increases in inflammatory markers and pro-inflammatory cytokines may lead to a deficit in serotonin and melatonin levels, which is an emerging theory on the etiology of depression (Gałecki and Talarowska, 2018). In the present study, although MPO activity levels (as a sign of neutrophil infiltration) were found to be increased with CMS, the lipid peroxidation levels (a measure of oxidative stress) and the pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) were not shown to be significantly changed. Taken together with the histological changes, these results suggest chronic inflammatory response but not acute inflammatory mediators are related to CMS-induced behavioral changes.

Regular exercise is known to be anti-inflammatory in depressive disorders (Schuch et al., 2016). Consistent with the literature, we found MPO activity and MDA levels were suppressed with CE, and for the first time, we show this is true for WW as well. Moreover, TNF-α levels were inhibited *via* both exercise models and IL-6 levels were suppressed by WW. Our results underline the anti-inflammatory role exercise *via* inhibiting neutrophil infiltration, lipid peroxidation, and pro-inflammatory cytokine responses and is consistent with previously reported effects of WW (Cakır et al., 2010).

CONCLUSION

In conclusion, our results demonstrate that CMS results in increased anhedonia, impaired cognition, increased anxiety levels, and induces a chronic inflammatory state. The related neurodegeneration may involve increased neutrophil migration and depleted antioxidant capacity in the brain. Furthermore, our data show WW is similarly protective in CMS-induced cognitive, inflammatory, and neurodegenerative deficits. We conclude for those who are not able to engage in regular exercise, a 'Weekend Warrior' exercise schedule can provide comparable protective and beneficial effects in combating cognitive and inflammatory insults caused by depression.

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