Physical exercise ameliorates psychiatric disorders and cognitive dysfunctions by hippocampal mitochondrial function and neuroplasticity in post-traumatic stress disorder

Jin-Hee Seo, Hye-Sang Park, Sang-Seo Park, Chang-Ju Kim, Dong-Hyun Kim, Tae-Woon Kim

A R T I C L E   I N F O

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A B S T R A C T

Post-traumatic stress disorder (PTSD) is a stress-related condition that can be triggered by witnessing or experiencing a life-threatening event, such as a war, natural disaster, terrorist attack, major accident, or assault. PTSD is caused by dysfunction of the hippocampus and causes problems associated with brain functioning, such as anxiety, depression, and cognitive impairment. Exercise is known to have a positive effect on brain function, especially in the hippocampus. In this study, we investigated the effect of aerobic exercise on mitochondrial function and neuroplasticity in the hippocampus as well as behavioral changes in animal models of PTSD. Exposure to severe stress resulted in mitochondrial dysfunction in the hippocampus, including impaired Ca²⁺ homeostasis, an increase in reactive oxygen species such as H₂O₂, a decrease in the O₂ respiration rate, and overexpression of membrane permeability transition pore-related proteins, including voltage-dependent anion channel, adenine nucleotide translocase, and cyclophilin-D. Exposure to extreme stress also decreased neuroplasticity by increasing apoptosis and decreasing the brain-derived neurotrophic factor level and neurogenesis, resulting in increased anxiety, depression, and cognitive impairment. The impairments in mitochondrial function and neuroplasticity in the hippocampus, as well as anxiety, depression, and cognitive impairment, were all improved by exercise. Exercise-induced improvement of the brain-derived neurotrophic factor level in particular might alter mitochondrial function, neuroplasticity, and the rate of apoptosis in the hippocampus. Therefore, exercise might be an important non-pharmacological intervention for the prevention and treatment of the pathobiology of PTSD.

1. Introduction

Exposure to extreme events can trigger a persistent state of generalized anxiety. For example, soldiers who have returned from war may experience anxiety and depression and be at increased risk of suicide (Mevisen and de Jongh, 2010; Krysinska and Lester, 2010). Anxiety disorders may also develop in response to other psychologically stressful environments and life events (Bremner, 1999; Broschot et al., 2018). Collectively, these disorders are known as post-traumatic stress disorder (PTSD). Psychiatric manifestations of PTSD include depression and panic attacks, and symptoms can include self-harm, suicidal thoughts, violent or antisocial behavior, and insomnia. Common physical manifestations include chronic gastrointestinal symptoms, migraine headaches, and nervous rashes that interfere with normal day-to-day life. There is a strong neurobiological correlation between PTSD and decreased hippocampal volume. The hippocampus is an important area in the limbic system of the brain that distinguishes between dangerous and safe situations. It is particularly sensitive to morphologic changes caused by stress (Rauch et al., 2006; McEwen, 2007; Woon et al., 2010). Dysfunction or impairment of the hippocampus is one of the causes of stress-related psychiatric disorders, such as PTSD (Adami et al., 2006). The hippocampus is known to control...
cognitive functioning and memory, especially of anxiety and fear (Rauch and Shin, 1997; Adami et al., 2006; Liberzon and Martis, 2006), and to store emotion-related information, including long-term memories (Hughes and Shin, 2011). Trauma and PTSD are closely associated with hippocampal impairment (Schacter, 1997; Eichenbaum, 2000; Corcoran and Maren, 2001; Megaugh, 2004; Shin et al., 2006; Schoenfeld et al., 2019). Moreover, mitochondrial dysfunction has recently been found to be involved in the pathology of PTSD (Su et al., 2008; Hauger et al., 2012; Li et al., 2013; Xing et al., 2013). The mitochondria are essential for neurotransmission, long-term and short-term neuroplasticity, cell elasticity in response to stress, and behavioral adaptation (Mattson et al., 2008; Quiroz et al., 2008; Jonas, 2009; MacAskill et al., 2010; Gleichmann and Mattsson, 2011). Many illnesses, including psychiatric disorders, involve functional impairment of the mitochondria.

There is a suggestion that chronic stress suppresses the mitochondrial respiratory chain in the brain (Madrigal et al., 2001). Mitochondria functional impairment and biochemical damage to the mitochondrial electron transport system are thought to be pathological causes of bipolar disorder and depression (Rezin et al., 2009). Functional impairment of the mitochondria may involve apoptosis or neurodegeneration in response to reactive oxygen species (ROS). The mitochondrion is the main controller of neurotransmitter signals in dendrites and synapses and has an important role in the functioning of neurons, including in ATP production, formation of synaptic proteins, lipid synthesis, intracellular calcium buffering, and recovery and death of cells (Jonas, 2009; Zündorf and Reiser, 2011). Mitochondrial impairment affects neuroplasticity, which can exacerbate emotional and psychiatric disorders (Manji et al., 2012). In contrast, physical exercise can bring about neural and biochemical changes in the brain that lead to improved mental and physical health by reducing stress and anxiety. Physical activity and exercise activate neurotransmitters and modulate brain function, thereby contributing to growth and survival of neurons (Stummer et al., 1994; Alkadhi, 2018), preserving neuroplasticity, and increasing mitochondrial activity in the brain (Cotman and Berchtold, 2002; Steiner et al., 2011). Therefore, it is thought that the behavioral changes that occur in PTSD, such as anxiety, depression, and cognitive impairment, occur in response to functional impairment of neuroplasticity in the brain because of mitochondrial dysfunction in the hippocampus. The aim of this study was to test the hypothesis that exercise can improve psychiatric status and cognitive functioning by increasing hippocampal mitochondrial function and neuroplasticity in a rat model of PTSD.

2. Materials and methods

2.1. Animals

All animal experiments were performed in accordance with the guidelines of the National Institutes of Health and the Korean Academy of Medical Science. The study protocol was approved by the Kyung Hee University Institutional Animal Care and Use Committee (approval number KHUASP [SE]-15-089). The rats were housed under conditions of controlled temperature (25 ± 1 °C) and lighting (7 am to 7 pm) with food and water ad libitum. Sixty 4-week-old male Sprague Dawley rats were randomly divided into a control (CON) group, a control and exercise (CON + EX) group, a PTSD group, and a PTSD and exercise (PTSD + EX) group (n = 15 in each group).

2.2. Exercise protocol

The exercise groups exercised on a treadmill made for animal use once daily on six days per week for 4 consecutive weeks. The exercise consisted of 5 min of warm-up at an inclination of 0° and a rate of 3 m/min, 30 min of main exercise at 10 m/min, and 5 min of cool-down at 3 m/min for the first 2 weeks. The rats then performed 40 min of main exercise at a rate of 12 m/min in week 3 and 50 min of main exercise at 13 m/min in week 4. No electric shocks were applied during treadmill exercise to minimize stress.

2.3. Animal model of PTSD

Animals in the PTSD groups received a total of 15 inescapable intermittent isolated electric shocks (intensity, 1 mA; interval, 10 s; duration, 10 s) that were delivered through the grid floor using a modification of the method described by Li et al. (2006). Briefly, each animal received inescapable electric foot shocks for 7 days and were then re-exposed to the same chamber without foot shocks on 3 days (days 2, 4, and 7). After 1 min of adaptation, each animal received an inescapable foot shock delivered over 5 min. The control animals were placed in the same chamber for 5 min without foot shock. We then observed the freezing behavior of the rats that had received inescapable electric shock on days 2, 4, and 7.

2.4. Behavioral tests

2.4.1. Open field

Locomotion and depressive-like behavior were evaluated using the open field test (OFT). The animals were randomly assigned to order of testing and placed in a square black open field area (77 cm × 77 cm × 25 cm) made of wood. The experimental area was enclosed by walls that were 40 cm high. Each animal was placed in the center of the exercise area and left to explore the environment freely for 1 min. Dependent measures were the distance traveled in the center and the total distance traveled during 5 min. The data were automatically collected via the Smart Video Tracking System (Smart version 2.5, Panlab, Barcelona, Spain).

2.4.2. Elevated plus maze

The elevated plus maze (EPM) apparatus consisted of two open arms (45 cm × 10 cm), crossed by two opposing arms of the same size. The junction area of the central measured platform (10 × 10 cm) was set up 65 cm above the floor. Each rat was placed on the central platform facing a closed arm and allowed to freely explore the maze for 5 min. Entry into an arm was defined as entry of all four paws into the arm. The number of entries and amount of time spent in the open arms were measured as an assessment of anxiety. The data were automatically collected via the Smart Video Tracking System (Smart version 2.5, Panlab, Barcelona, Spain).

2.4.3. Forced swimming

The forced swimming test (FST) was used to confirm a depressive state. The experimental animals were placed individually in glass cylinders (height, 50 cm; diameter, 15 cm) containing water at a temperature of 25 °C and a depth of approximately 30 cm. All rats underwent a 15-min pretest to confirm their adaptation to water. One day after the pretest, the animals were tested for 5 min. During the test session, periods of immobility and climbing were measured using the Smart version 2.5 Video Tracking System. Climbing behavior consisted of upward-directed movements of the forepaws along the side of the swim chamber. Immobility was defined to occur when no additional activity was observed other than the actions needed by the animal to keep its head above the water.

2.4.4. Morris water maze

The Morris water maze task was used to assess spatial learning and working memory. One day before training, the rats were habituated to swimming for 60 s in the pool without a platform. All rats were trained three times a day for 5 consecutive days. A probe trial was performed 24 h after the final training session. When a rat found the platform, it was allowed to remain there for 30 s. If the rat did not find the platform within 60 s, it was guided by hand to the platform. The rats underwent
a 60-s retention probe test, after which the platform was removed from the pool. The data were automatically collected by the Smart Video Tracking System.

2.5. Preparation of tissue

The rats were euthanized immediately after the behavior test. To prepare the brain slices, the animals were fully anesthetized with ethyl ether, perfused transcardially with 50 mM phosphate-buffered saline (PBS), and then fixed with a freshly prepared solution of 4% paraformaldehyde in 100 mM phosphate buffer (pH 7.4). The brains were then removed, post-fixed in the same fixative overnight, and transferred into a 30% sucrose solution for cryoprotection. Coronal sections with a thickness of 40 μm were created using a freezing microtome (Leica, Nussloch, Germany).

2.6. Isolation of hippocampal mitochondria

Mitochondria were isolated from the rat brain with a Mitochondria Isolation Kit for Tissue (Thermo #89801; Thermo Fisher Scientific, Waltham, MA) using a reagent-based method according to the manufacturer's instructions. Briefly, homogeneous suspensions of hippocampal tissue were prepared using a pre-chilled homogenizer (T 10 basic Ultra-Turrax®, Ika, Staufen im Breisgau, Germany). The hippocampal suspensions were spun at 1000 × g for 5 min at 4 °C. The pellet was suspended in 800 μl of bovine serum albumin/reagent A solution and incubated for 2 min before adding 10 μl of isolation reagent B. After incubating for 5 min with intermittent vortexing, 800 μl of reagent C was added. The resulting cell lysate was centrifuged at 700 × g for 10 min at 4 °C, after which the supernatant was transferred to a new tube and spun at 30000 × g for 15 min at 4 °C. The supernatant (cytosolic fraction) was collected for analysis. After washing with mitochondrial isolation reagent C, the mitochondrial pellet was lysed with 2% CHAPS in Tris-buffered saline containing protease inhibitors. The samples were stored at −80 °C until use.

2.7. Immunoﬂuorescence for BrdU/NeuN and DAPI/DCX

BrdU/NeuN-positive and DAPI/DCX-positive cells in the dentate gyrus were tested for immunofluorescence. In brief, the brain sections were permeabilized by incubation in 0.5% Triton X-100 in PBS for 20 min, incubated in 50% formamide-2 × standard saline citrate at 65 °C for 2 h, denatured in 2 N HCl at 37 °C for 30 min, and rinsed twice in 100 mM sodium borate (pH 8.5), in that order. The sections were incubated overnight with rat anti-BrdU antibody (1:500; Abcam, Cambridge, UK), mouse anti-NeuN antibody (1:500; Millipore, Temecula, CA), and rabbit anti-DCX (1:500; Abcam). The brain sections were then washed in PBS and incubated with the appropriate secondary antibodies for 2 h. The secondary antibodies used were anti-mouse IgG Alexa Fluor-488, anti-rabbit IgG Alexa Fluor-594, and anti-rat IgG Alexa Fluor-550. The DNA was stained with DAPI (Santa Cruz Biotechnology, San Diego, CA) and mounted on glass slides. Images were captured using an FV10i FLuoview confocal microscope (Olympus, Tokyo, Japan).

2.8. TUNEL staining

To visualize DNA fragmentation, we performed TUNEL staining using an In Situ Cell Death Detection Kit (Roche Diagnostics, Risch-Rotkreuz, Switzerland) according to the manufacturer’s protocol. Sections were post-fixed in ethanol-acetic acid (2:1), rinsed, incubated with proteinase K (100 mg/ml) and then rinsed again. Next, they were incubated in 3% H2O2, permeabilized with 0.5% Triton X-100, rinsed again, and incubated in the TUNEL reaction mixture. The sections were rinsed and visualized using Converter-POD with 0.03% DAB, counter-stained with Nissl, and mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature and cover-slipped using Permount mounting medium.

2.9. Western blotting for BDNF, TrkB, Bax, Bcl-2, cytochrome c, Apaf-1, cleaved caspases-3/-9, VDAC1, ANTI/2, and Cyp-D

The hippocampal tissues were homogenized on ice and lysed in a lysis buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 0.5% deoxycholic acid, 1% Nonidet P40, 0.1% sodium dodecyl sulfate, 1 mM PMSF, and leupeptin 100 mg/ml. The protein content was measured using a colorimetric protein assay kit (Bio-Rad, Hercules, CA). Thirty micrograms of protein were separated on sodium dodecyl sulfate-polyacrylamide gels and transferred onto a nitrocellulose membrane, which was incubated with mouse β-actin antibody (1:1000; Santa Cruz Biotechnology), mouse Bax, Bcl-2 and cytochrome c (1:1000; Santa Cruz Biotechnology), and COX IV (1:1000; Cell Signaling, Beverly, MA), brain-derived neurotrophic factor (BDNF; 1:1000; Bioss Inc., Woburn, MA), TrkB (1:1000; Santa Cruz Biotechnology), Apaf-1 (1:1000; Cell Signaling), cleaved caspase-9 (1:500; Cell Signaling), cleaved caspase-3 (1:700; Cell Signaling), voltage-dependent anion channel-1 (VDAC1; 1:500; Bioss Inc.), adenine nucleotide translocase (ANT1/2; 1:1000; Proteintech Group Inc., Chicago, IL), and cyclophilin-D (Cyp-D, 1:1000; Thermo Fisher). Horseradish peroxidase-conjugated anti-mouse for Bax, Bcl-2, cytochrome c, and β-actin, and anti-rabbit for BDNF, TrkB, Apaf-1, cleaved caspase-3, cleaved caspase-9, VDAC1, ANTI/2, and Cyp-D were used as secondary antibodies.

2.10. Mitochondrial Ca2+ retention capacity

The mitochondrial calcium retention capacity was tested to assess the susceptibility of the mitochondrial permeability transition pore (mPTP) to opening. Briefly, after grinding the hippocampal tissue, overlaid traces of changes in fluorescence induced by Calcium Green-5 N were measured continuously (ΔF/min) at 37 °C during state 4 respiration using a Spex Fluoromax 4 spectrofluorometer (Horiba Scientific, Edison, NJ). After establishing the background ΔF (hippocampal tissue in the presence of 1 μM Calcium Green-5 N, 1 μM hexokinase, 0.04 mM EGTA, 1.5 mM thapsigargin, 5 mM 2-deoxyglucose, 5 mM glutamate, 5 mM succinate, and 2 mM malate), the reaction was initiated by addition of Ca2+ pulses (12.5 mM), with excitation and emission wavelengths set at 506 nm and 532 nm, respectively. The total mitochondrial Ca2+ retention capacity prior to PTP opening (i.e., release of Ca2+) was expressed as pmol/mg wet tissue weight.

2.11. Mitochondrial O2 respiration

Mitochondrial O2 consumption was measured by polarographic high-resolution respirometry (O2K Oxygraph, Oroboros, Innsbruck, Austria) at 30 °C in assay respiration buffer (Buffer Z added to 20 mM creatine and 50 μM EGTA) using the following protocol: (i) 5 mM glutamate (complex I substrate) + 2 mM malate (complex I substrate), (ii) 4 mM ADP (state 3 condition), and (iii) 10 mM succinate. Mitochondrial O2 respiration was then normalized by the wet tissue weight and was expressed in pmol/s/mg wet tissue weight.

2.12. Mitochondrial H2O2 emission

H2O2 emission was measured at 37 °C (ΔF/min) during state 4 respiration (10 μg/ml oligomycin) by continuously monitoring oxidation of Amplex Red (excitation/emission λ = 563/587 nm) using a Spex Fluoromax 4 spectrofluorometer with the following protocol: 10 μM Amplex Red 1 U/ml horseradish peroxidase 10 μg/ml oligomycin) settings, and 1 mM malate + 2 mM glutamate (complex I substrates), 3 mM succinate (complex II substrate) and 10 mM glycero-3-phosphate (lipid substrate). The H2O2 emission rate after removing the
background value from each of the standard values (standard curve) was calculated from the ΔF/min gradient values and expressed as pmol/min/mg wet tissue weight.

2.13. Statistical analysis

The data were analyzed using two-way analysis of variance (PTSD [factor A; non-PTSD vs. PTSD] and exercise [factor B; non-exercise vs. exercise]). Post hoc testing was conducted by pairwise comparisons. Spatial learning was measured by two-way mixed analysis of variance to account for repeated measures (4 groups; between × 5 day repeated; within). Post hoc analysis was performed using pairwise comparisons. α = 0.05 was considered statistically significant.

3. Results

3.1. Effects of treadmill exercise on anxiety, depression, spatial learning, and memory

There were significant interaction effects of number of entries and time spent in the open arms between PTSD (factor A) and exercise (factor B) (number of entries into open arms: M = 40.933, SD = 7.648, F1,56 = 6.7, p = .012; time spent in open arms; M = 77.2, SD = 26.546, F1,56 = 8.162, p = .006). Post hoc analysis revealed a significant difference between the CON and PTSD groups and between the PTSD and PTSD + EX groups (number of entries into open arms: MD = 17.267, SE = 4.589, p = .000; time spent in open arms: MD = 44.067, SE = 11.056, p = .000; Fig. 1A–C). On the FST, there was no significant interaction effect of immobility time between PTSD and exercise (M = 77.45, SD = 19.746). There was a significant difference in the main effect of PTSD between the non-PTSD (CON and CON + EX) and PTSD (PTSD and PTSD + EX) groups (MD = 49.498, SE = 7.051, F1,56 = 49.278, p = .000) and exercise between the non-exercise (CON and PTSD) and exercise (CON + EX and PTSD + EX) groups (MD = 22.985, SE = 7.051, F1,56 = 10.626, p = .002; Fig. 1D). On the OFT, there was no significant interaction effect of open field distance from the center between PTSD and exercise (M = 110.0, SD = 26.621). There was a significant difference in the main effect of PTSD (MD = 49.133, SE = 9.8, F1,41 = 25.138, p = .000) and exercise (MD = 37.4, SE = 9.8, F1,41 = 14.566, p = .000; Fig. 1E, F). Total distance was no significant interaction effect (data not shown). The experience of PTSD had a negative impact on anxiety and depression that was attenuated by exercise. On the Morris water maze task, spatial learning was measured as the time spent on the platform on three occasions daily for 5 days. In the two-way mixed analysis of variance of spatial learning, there was no interaction effect of group and day (repeated measures). Post hoc analysis revealed a significant difference in the main effect of group (MD = 6.4, SE = 2.127, F8, 41 = 3.456, p = .019) and the main effect of day was significantly different in all each other days except between days 1 and 2 (F4, 113 = 108.801, p = .000; Fig. 1G). Working memory was evaluated as the latency until the main effect of PTSD (MD = 186.915, SE = 23.64, F1,36 = 62.516, p = .000) and exercise (MD = 144.725, SE = 23.64, F1,36 = 37.479, p = .000; Fig. 2A, B). Using the mitochondrial Ca2+ retention capacity in the CON group as a baseline (i.e., 100%), we observed a significant interaction effect of membrane PTP sensitivity between PTSD and exercise (M = 113.815, SD = 10.624, F1,36 = 74.156, p = .000). Post hoc analysis revealed a significant difference between the CON and PTSD groups (MD = 144.798, SE = 8.806, F1,36 = 270.404, p = .000), CON + EX and PTSD + EX groups (MD = 37.561, SE = 8.806, F1,36 = 18.196, p = .000), CON and CON + EX groups (MD = 23.745, SE = 8.806, F1,36 = 7.272, p = .011), PTSD and PTSD + EX groups (MD = 130.982, SE = 8.806, F1,36 = 221.265, p = .000; Fig. 2C). The negative impact of PTSD on mitochondrial Ca2+ retention and membrane PTP sensitivity was ameliorated by exercise. Mitochondrial membrane-associated proteins were investigated to assess membrane PTP. There was a significant interaction effect of VDAC1 between PTSD and exercise (M = 1.054, SD = 0.162, F1,36 = 9.425, p = .004). Post hoc analysis revealed a significant difference between the CON and PTSD groups (MD = 0.356, SE = 0.078, F1,36 = 20.885, p = .000) and between the PTSD and PTSD + EX groups (MD = 0.301, SE = 0.078, F1,36 = 14.962, p = .000; Fig. 2D). And there was no significant interaction effect of ANT1/2 or Cyp-D between PTSD and exercise (ANT1/2; M = 1.7222, SD = 0.522, Cyp-D; M = 1.271, SD = 0.289). There were significant differences in the main effects of PTSD (ANT1/2, MD = 1.074, SE = 0.117, F1,36 = 84.293, p = .000; Cyp-D, MD = 0.5, SE = 0.074, F1,36 = 45.514, p = .000) and exercise (ANT1/2, MD = 0.351, SE = 0.117, F1,36 = 9.030, p = .005; Cyp-D, MD = 0.229, SE = 0.074, F1,36 = 9.522, p = .004; Fig. 2E, F). These results indicate that PTSD increased membrane PTP opening sensitivity by overexpression of VDAC1, ANT1/2, and Cyp-D that exercise stabilized PTP opening by increasing protein levels in the mitochondrial membrane of the hippocampus.

3.2. 3.3. Effects of exercise on mitochondrial O2 respiration and H2O2 emission in the hippocampus

The mitochondrial O2 respiration protocol included basal, glutamate-, and malate-supported respiration (electron entry via complex I; state 2, GM2), ADP-supported respiration (state 3, GM3), and succinate-supported respiration (electron entry via complex II; state 3, GMS3). With respect to GM3 respiration, there was a significant interaction effect of PTSD and exercise (M = 4.121, SD = 0.178, F1,36 = 77.612, p = .000). In post hoc analysis, there were significant differences between the CON and PTSD groups (MD = 1.253, SE = 0.219, F1,36 = 32.863, p = .000), CON + EX and PTSD + EX groups (MD = 3.977, SE = 0.219, F1,36 = 330.932, p = .000), and CON and CON + EX groups (MD = 3.381, SE = 0.219, F1,36 = 239.155, p = .000), and PTSD and PTSD + EX groups (MD = 0.657, SE = 0.219, F1,36 = 9.034, p = .005). There was no significant interaction effect of GM2 or GMS3 between PTSD and exercise (GM2, M = 0.986, SD = 0.099; GMS3, M = 15.428, SD = 1.165). There was a significant difference in the main effect of PTSD (GM2, MD = 0.825, SE = 0.094, F1,36 = 76.897, p = .000; GMS3, MD = 4.859, SE = 0.346, F1,36 = 197.236, p = .000) and exercise (GM2, MD = 0.708, SE = 0.094, F1,36 = 56.662, p = .000; GMS3, MD = 3.054, SE = 0.346, F1,36 = 77.906, p = .000; Fig. 3A, B). The mitochondrial H2O2 emission rate was calculated in the complex-I substrate (glutamate + malate, GM), the complex-2 substrate (GM + succinate, GMS), and lipid substrate (GMS + glyceral-3-phosphate, GMSG3P). There were significant interaction effects of emission of H2O2 GMS and H2O2 GMSG3P between PTSD and exercise (H2O2 GMS, M = 9.805, SD = 2.291, F1,36 = 7.304, p = .010; H2O2 GMSG3P, M = 12.446, SD = 1.549, F1,36 = 16.689, p = .000). The post hoc analysis identified a significant difference between the CON and PTSD groups (H2O2 GMS, MD = 8.035, SE = 1.484, F1,36 = 29.308, p = .000; H2O2 GMSG3P, MD = 11.965, SE = 1.805, F1,36 = 43.929, p = .000) and between the PTSD and PTSD + EX groups (H2O2 GMS, MD = 6.722, SE = 1.484, F1,36 = 20.513, p = .000; H2O2 GMSG3P, MD = 11.364,
SE = 1.805, \( F_{1,36} = 39.624, p = .000 \). There was no significant interaction effect of H\(_2\)O\(_2\) GM expression between PTSD and exercise (\( M = 1.363, SD = 0.314 \)). There was a significant difference in the main effect of PTSD (\( MD = 0.341, SE = 0.135, F_{1,36} = 6.389, p = .016 \)) and exercise (\( MD = 0.308, SE = 0.135, F_{1,36} = 5.223, p = .028 \); Fig. 3C, D). These results indicate that PTSD had negative effects, including a decrease in production of ATP and an increase in ROS that was improved by the effects of exercise on \( O_2 \) respiration and \( H_2O_2 \) emission in the hippocampal mitochondria.

### 3.4. Effects of treadmill exercise on Bax, Bcl-2, cytochrome c, Apaf-1, and cleaved caspase-3/-9 in the hippocampal mitochondria and cytosol, and TUNEL-positive cells in the dentate gyrus

TUNEL-positive assays for mitochondrial Bax, Bcl-2, cytochrome c and for cytosolic Bax, Bcl-2, cytochrome c, Apaf-1, and cleaved caspase-3/-9 were used to evaluate apoptosis in the dentate gyrus. The mitochondrial and cytosolic levels in the CON group were set at 1.00. There were significant interaction effects of mitochondrial Bax expression (\( M = 1.516, SD = 0.097, F_{1,36} = 28.282, p = .000 \); Fig. 4A) and cytosolic Bcl-2 (\( M = 1.313, SD = 0.163, F_{1,36} = 8.008, p = .008 \); Fig. 4E), Apaf-1 (\( M = 1.212, SD = 0.303, F_{1,36} = 6.416, p = .016 \); Fig. 4G), cleaved caspase-9 (\( M = 1.222, SD = 0.421, F_{1,36} = 12.674, p = .001 \); Fig. 4H), and cleaved caspase-3 (\( M = 1.374, SD = 0.649, F_{1,36} = 9.114, p = .005 \); Fig. 4I) between PTSD and exercise. Post hoc analysis revealed a significant difference between the CON and PTSD groups (mitochondrial Bax, \( MD = 1.416, SE = 0.130, F_{1,36} = 118.591, p = .000 \); cytosolic Bcl-2, \( MD = 0.479, SE = 0.064, F_{1,36} = 56.795, p = .000 \); cytosolic Apaf-1, \( MD = 0.798, SE = 0.129, F_{1,36} = 38.425, p = .000 \); cytosolic cleaved caspase-9, \( MD = 1.415, SE = 0.186, F_{1,36} = 57.980, p = .000 \); cytosolic cleaved caspase-3, \( MD = 1.382, SE = 0.191, F_{1,36} = 52.399, p = .000 \), between the CON...
+EX and PTSD+EX groups (mitochondrial Bax, MD = 0.438, SE = 0.130, F_{1,36} = 11.350, p = .002; cytosolic Bcl-2, MD = 0.224, SE = 0.064, F_{1,36} = 12.492, p = .001; cytosolic Bax, MD = 0.337, SE = 0.129, F_{1,36} = 6.846, p = .013; cytosolic cleaved caspase-9, MD = 0.479, SE = 0.186, F_{1,36} = 6.655, p = .014; cytosolic cleaved caspase-3, MD = 0.567, SE = 0.191, F_{1,36} = 8.817, p = .005), and between the PTSD and PTSD+EX groups (mitochondrial Bax, MD = 0.899, SE = 0.130, F_{1,36} = 47.823, p = .000; cytosolic Bcl-2, MD = 0.166, SE = 0.064, F_{1,36} = 6.801, p = .013; cytosolic Apaf-1, MD = 0.586, SE = 0.129, F_{1,36} = 20.722, p = .000; cytosolic cleaved caspase-9, MD = 1.192, SE = 0.186, F_{1,36} = 41.155, p = .000; cytosolic cleaved caspase-3, MD = 1.008, SE = 0.191, F_{1,36} = 27.859, p = .000). There was no significant interaction effect of mitochondrial Bcl-2, cytosolic Bax, and mitochondrial and cytosolic cytochrome c expression levels between PTSD and exercise (mitochondrial Bcl-2, MD = 0.291, SE = 0.084, F_{1,36} = 12.080, p = .001; mitochondrial cytochrome c, MD = 0.291, SE = 0.084, F_{1,36} = 12.080, p = .001; mitochondrial Bcl-2, MD = 0.302, SE = 0.084, F_{1,36} = 12.977, p = .001; mitochondrial cytochrome c, MD = 0.302, SE = 0.084, F_{1,36} = 12.977, p = .001; mitochondrial Bcl-2, MD = 1.192, SE = 0.186, F_{1,36} = 230.081, p = .000; mitochondrial cytochrome c, MD = 1.192, SE = 0.186, F_{1,36} = 230.081, p = .000; mitochondrial Bcl-2, MD = 0.166, SE = 0.064, F_{1,36} = 6.801, p = .013; mitochondrial cytochrome c, MD = 0.166, SE = 0.064, F_{1,36} = 6.801, p = .013; mitochondrial Bcl-2, MD = 0.586, SE = 0.129, F_{1,36} = 20.722, p = .000; mitochondrial cytochrome c, MD = 0.586, SE = 0.129, F_{1,36} = 20.722, p = .000; mitochondrial Bcl-2, MD = 1.008, SE = 0.191, F_{1,36} = 27.859, p = .000; mitochondrial cytochrome c, MD = 1.008, SE = 0.191, F_{1,36} = 27.859, p = .000; mitochondrial Bcl-2, MD = 1.192, SE = 0.186, F_{1,36} = 41.155, p = .000; mitochondrial cytochrome c, MD = 1.192, SE = 0.186, F_{1,36} = 41.155, p = .000; mitochondrial Bcl-2, MD = 1.973, SE = 0.101, F_{1,36} = 112.668, p = .000; mitochondrial cytochrome c, MD = 1.973, SE = 0.101, F_{1,36} = 112.668, p = .000). There was a significant difference in the main effect of PTSD (mitochondrial Bcl-2, MD = 0.291, SE = 0.084, F_{1,36} = 12.080, p = .001; mitochondrial cytochrome c, MD = 0.644, SE = 0.048, F_{1,36} = 183.282, p = .000; cytosolic Bax, MD = 0.166, SE = 0.064, F_{1,36} = 6.801, p = .013; cytosolic cytochrome c, MD = 0.166, SE = 0.064, F_{1,36} = 6.801, p = .013; cytosolic Bax, MD = 0.586, SE = 0.129, F_{1,36} = 20.722, p = .000; cytosolic cytochrome c, MD = 0.586, SE = 0.129, F_{1,36} = 20.722, p = .000; cytosolic Bax, MD = 1.008, SE = 0.191, F_{1,36} = 27.859, p = .000; cytosolic cytochrome c, MD = 1.008, SE = 0.191, F_{1,36} = 27.859, p = .000; Fig. 4B–D, 4F). TUNEL staining revealed a significant interaction effect between PTSD and exercise (M = 18.125, SD = 10.793, F_{1,36} = 7.869, p = .008). Post hoc analysis revealed a significant difference between the CON and PTSD groups (M = 26.481, SD = 3.447, F_{1,36} = 59.029, p = .000), CON+EX and PTSD+EX groups (M = 12.808, SE = 3.447, F_{1,36} = 13.809, p = .001), and PTSD and PTSD+EX groups (M = 13.899, SE = 3.447, F_{1,36} = 16.261, p = .000; Fig. 4J, K). This...
indicates that exercise had a greater impact in the PTSD group than in the other groups. PTSD had an influence in all groups and exercise attenuated mitochondrial Bax, cytosolic Bcl-2, Apaf-1, and cleaved caspase-3/−9, and TUNEL-positive cells. The experience of PTSD and exercise affected expression levels of mitochondrial Bcl-2 and cytochrome c and of cytosolic Bax and cytochrome c. These results indicate an association of PTSD with apoptosis of mitochondrial and cytosolic cells in the hippocampus.

3.5. Effects of treadmill exercise on neurogenesis and cell differentiation in the dentate gyrus

We evaluated BrdU/NeuN-positive and DCX-positive cells in the dentate gyrus, for which staining indicates neurogenesis and cell differentiation, respectively. There was no significant interaction effect of BrdU/NeuN or DCX positivity between PTSD and exercise (BrdU/NeuN, MD = 36.2, SE = 10.115, $F_{1,36} = 12.808, p = .001$; DCX, MD = 175.39, SE = 25.943, $F_{1,36} = 45.707, p = .000$) and exercise (BrdU/NeuN, MD = 34.442, SE = 10.115, $F_{1,36} = 11.594, p = .002$; DCX, MD = 161.368, SE = 25.943, $F_{1,36} = 38.690, p = .000$; $p < .05$, a statistically significant main effect of PTSD (factor A). $p < .05$, a statistically significant main effect of exercise (factor B). $p < .05$, a statistically significant between-group difference because of the interaction effect. CON, control; CON+EX, control plus exercise; PTSD, post-traumatic disorder; PTSD+EX, PTSD plus exercise.

3.6. Effects of treadmill exercise on hippocampal BDNF and TrkB expression

Changes in hippocampal expression of neurotrophic factor and its receptor were assessed by measuring BDNF and TrkB levels. The BDNF and TrkB levels in the CON group were set to 1.00. There was a significant interaction effect of BDNF expression between PTSD and exercise ($M = 0.878, SD = 0.090, F_{1,36} = 6.796, p = .013$). Post hoc analysis revealed a significant difference between the CON and PTSD groups ($MD = 0.383, SE = 0.062, F_{1,36} = 38.304, p = .000$), PTSD and PTSD+EX groups ($MD = 0.261, SE = 0.062, F_{1,36} = 17.813, p = .000$), CON+EX and PTSD+EX groups ($MD = 0.155, SE = 0.062, F_{1,36} = 6.262, p = .017$; Fig. 6 left). There was no significant interaction effect of TrkB expression between PTSD and exercise ($M = 1.025, SD = 0.061$). The main effect of exercise showed only TrkB ($MD = 0.075, SE = 0.027, F_{1,36} = 7.453, p = .01; Fig. 6 right). Therefore, the experience of PTSD affected BDNF expression and exercise attenuated expression of both BDNF and TrkB.
4. Discussion

PTSD is a stress-related disorder caused by witnessing or experiencing life-threatening events that are relived in nightmares and flashbacks. These symptoms can be long-lasting and render affected individuals vulnerable to anxiety-related and stress-related psychopathology (Sipos et al., 2014). In our study, animals with PTSD showed behavioral symptoms of depression and anxiety, and decreases in spatial learning and memory. Previous studies have reported that animals with PTSD show significant decreases in time spent in and distance from the center on the OFT, in time spent and entries into the open arms on the EPM, in anxiety and spatial learning and memory on the MWM, and increased immobility time on the FST, in depression (Wang et al., 2009; Serova et al., 2013; Ji et al., 2016; Zhou et al., 2019). Anxiety, in particular, is inextricably linked with loss of memory. Anxiety both an indirect and direct predictor of future cognitive impairment (Sinoff and Werner, 2003), and PTSD is associated with impaired learning and memory. This cognitive impairment has been suggested to be affected by the severity and frequency of PTSD-related depression (Burriss et al., 2008). The hippocampus plays an important role in the onset of psychiatric illness and the impaired learning and memory associated with PTSD. Exposure to severe stress damages the hippocampus. Previous research suggests a close relationship between stress-related psychiatric illness and decreased hippocampal volume (Gilbertson et al., 2002; Rauch et al., 2006; McEwen, 2007; Woon et al., 2010). Structural and functional changes in the hippocampus are related to the pathobiology of PTSD (Hull, 2002; Hughes and Shin, 2011), particularly changes in the mitochondria. Genetic and environmental factors can interfere with the functioning of the mitochondria, e.g., ATP production, buffering of Ca2+ in the cytoplasm, production of ROS, cellular respiration, and mitochondrial division and fusion. This may result in stress-related abnormal biological responses that can increase the risk of PTSD and contribute to its symptomatology (Preston et al., 2018). Impaired mitochondrial function can result in excessive superoxide and H2O2 levels, overproduction of ROS, and increased sensitivity of Ca2+ to mPTP (Anderson et al., 2011). Disruption of calcium homeostasis and increased mitochondrial levels of ROS, such as H2O2, result in increased sensitivity of mPTP opening, so play an important role in apoptosis and impede the respiratory chain and production of ATP (Kroemer and Reed, 2000; Brown, 2003; Kristian et al., 2007; Tsujimoto and Shimizu, 2007). Reduced mitochondrial respiration of oxygen is associated with a reduction in the functioning of the mitochondria, including decreased production of ATP (Heo et al., 2017). For example, decreases in mitochondrial respiratory capacity and ATP production and an increase in release of ROS in the brain has been reported in a rat model of anxiety (Hollis et al., 2015). In this study, the hippocampal mitochondria in the PTSD group showed decreased O2 respiration and Ca2+ retention and increased H2O2 levels, indicating decreased production of ATP, disruption of calcium-buffering homeostasis, and increased ROS levels. A decrease in mitochondrial Ca2+ retention is a sign of heightened mPTP sensitivity (Park et al., 2018).

The mitochondrial PTP complex consists of an outer membrane protein (VDAC), an inner membrane protein (ANT), and a mitochondrial matrix protein (Cyp-D). This pore mediates cell death by increasing the permeability of the inner mitochondrial membrane, promoting swelling of the matrix, rupture of the outer membrane, and release of cytochrome c (Brenner and Grimm, 2006). Increased production of ROS has been suggested to increase apoptosis by causing overexpression of VDAC, ANT-1, and Cyp-D (Bauer et al., 1999; Yuan et al., 2008; Ma et al., 2011). Overexpression of VDAC results in its oligomerization (Mc Commis and Baines, 2012). Overexpression of ANT-1 accelerates apoptosis, depletes ATP (Bauer et al., 1999), suppresses calcium uptake (Wieckowski et al., 2006), and depletes energy in the mitochondria, causing leak of ATP from the matrix and cell death (Chevrollier et al., 2011). Overexpression of Cyp-D is associated with
Fig. 5. Effects of exercise on BrdU/NeuN and DAPI/DCX staining in the dentate gyrus. Photomicrographs and data of BrdU/NeuN (A, B) and DAPI/DCX-positive cells (C, D). The scale bar represents 50 μm. The data are shown as the mean ± standard error of the mean (SEM). *p < .05, a statistically significant main effect of PTSD (factor A). #p < .05, a statistically significant main effect of exercise (factor B). CON, control; CON+EX, control plus exercise; PTSD, post-traumatic disorder; PTSD+EX, PTSD plus exercise.

Fig. 6. Effects of exercise on BDNF and TrkB protein expression in the hippocampus. Expression of BDNF and TrkB in the hippocampus. The data are shown as the mean ± standard error of the mean (SEM). #p < .05, a between-group difference because of the interaction effect. CON, control; CON+EX, control plus exercise; PTSD, post-traumatic disorder; PTSD+EX, PTSD plus exercise.
swelling of the mitochondria and spontaneous cell death (Baines et al., 2005). The inner, outer, and matrix mitochondria proteins are known to interact with each other (Kroemer and Reed, 2000).

Our study showed that overexpression of VDAC1, ANT1/2, and Cyc-D in the hippocampal mitochondria resulted in increased PTP sensitivity in the PTSD group. Moreover, Bax increased and Bel-2 and cytochrome c decreased in the mitochondria; in contrast, Bax decreased and Bel-2, cytochrome c, Apaf-1, and cleaved caspase-3/−9 and caspase-3 increased in the cytosol, resulting in increased TUNEL-positive cells in the dentate gyrus. Release of cytochrome c into the cytoplasm makes apoptosis irreversible. Cytosolic caspase-3 and caspase-9 trigger PTP opening during conditions of oxidative stress, which has a major role in the release of cytochrome c (De Giorgi et al., 2002). Furthermore, Bax, a pro-apoptotic factor, also activates the mitochondrial PTP and forms a transmembrane pore that is large enough to allow release of cytochrome c (Gómez-Crisóstomo et al., 2013). The functioning of the mitochondria determines not only the rate of cell death but also that of generation of new neurons. In our study, we observed increased levels of mitochondrial dysfunction and a decrease in cell differentiation and neurogenesis in the dentate gyrus in the PTSD group. A previous study also showed an association of functional impairment in the mitochondria and decreased neurogenesis in the hippocampus that could be improved by pharmacological intervention (Beckervordersandforth et al., 2017). Overall, the evidence suggests that the decreased hippocampal volume seen in PTSD is attributable to increased cell death and decreased neurogenesis-dependent hippocampal plasticity (Steib et al., 2014) and to improve calcium homeostasis, control of ROS production, and PTP sensitivity in the hippocampal mitochondria (Park et al., 2018). In particular, exercise has been shown to improve the redox balance in the hippocampus by upregulating antioxidants and decreased production of ROS (Marosi et al., 2012) possibly because it can stabilize overexpression of proteins such as VDAC, ANT, and Cyc-D, which are involved in opening of the PTP, and thereby decrease apoptosis. In our study, exercise decreased Bax, increased Bel-2, suppressed release of cytochrome c into the cytoplasm in the hippocampal mitochondria, and decreased the cell death rate. Bel-2 expression in particular increases mitochondrial Ca2+ uptake capacity and regulates oxidation, formation of free radicals, and the membrane potential in the mitochondrion. Furthermore, Bel-2 expression inhibits formation of Bax pores on the outer mitochondrial membrane, resulting in reduced release of Ca2+ and cytochrome c from the mitochondrion (Einat et al., 2005; McEwen et al., 2015). Activation of Bel-2 by exercise might increase the functioning of the mitochondria.

It is thought that the mitochondria are an important target for increased neurogenesis-dependent hippocampal plasticity (Steib et al., 2014) and that exercise may ameliorate emotional disorders by influencing mitochondria engagement and strengthening neural plasticity (Aguiar Jr et al., 2014). Exercise also increases the cell proliferation and neurogenesis in the hippocampus (van Praag et al., 1999; van Praag et al., 2005). In our study, the PTSD-related decrease in neurogenesis and cell differentiation in the dentate gyrus was improved by exercise. BDNF is involved in the control of neurogenesis, and exercise is known to activate BDNF in the hippocampus (Wran et al., 2013). Normal BDNF-TrkB signaling is vital for long-term survival of new neurons in the dentate gyrus (Sairanen et al., 2005) and can suppress activation of caspase-3 (Han et al., 2000). Moreover, the density of mitochondria in the brain has been shown to vary according to the BDNF level and to respond negatively to calcium overload in the mitochondria (Markham et al., 2004). While BDNF activation decreased in the PTSD group in our study, it increased with exercise. However, there was no difference in expression of TrkB, which is a BDNF receptor, between the control and PTSD groups; however, exercise increased expression of this protein in each group. Furthermore, a decrease in BDNF expression in the hippocampus has been shown to be correlated with stress-related depressive behavior (Duman and Monteggia, 2006).

5. Conclusions

PTSD-induced hippocampal dysfunction, particularly decreased neuroplasticity and increased apoptosis in response to impaired mitochondrial function, appears to be closely related to a decreased hippocampal volume in the presence of PTSD. These hippocampal impairments were associated with both cognitive impairment and symptoms of depression and anxiety. In contrast, exercise strengthened mitochondria in the hippocampus by increasing neuroplasticity, suppressing cell death, and ultimately improving the behavioral symptoms of PTSD. An exercise-induced increase in expression of BDNF may change the function, neuroplasticity, and apoptosis signals in the mitochondria of the hippocampus, thereby contributing to the prevention and treatment of PTSD. Physiological stimulation, such as exercise, in addition to pharmacologic treatment, could improve the outcomes in patients with PTSD.

Declaration of Competing Interest

The authors declare no conflict of interest.

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