HHS Public Access

Author manuscript

Physiol Behav. Author manuscript; available in PMC 2015 May 10.

Published in final edited form as:

Physiol Behav. 2014 May 10; 130: 135–144. doi:10.1016/j.physbeh.2014.04.011.

Novel mechanistic insights into treadmill exercise based rescue of social defeat-induced anxiety-like behavior and memory impairment in rats

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Abstract

Social defeat (SD) induced stress causes physiological and behavioral deficits in rodents, including depression and anxiety-like behaviors, as well as memory impairment. Anxiolytic and mood elevating effects of physical exercise are also known. However, rescue effect of physical exercise in social defeat-induced anxiety, depression or memory impairment has not been addressed. Role of epigenetic mechanisms that potentially contribute to these rescue or protective effects are also not known. Present study investigated the effect of moderate treadmill exercise on anxiety-like behavior and memory function in rats subjected to SD using a modified version of the resident-intruder model for social stress (defeat). Changes in histone acetylation and histone-modifying enzymes were examined in hippocampus, amygdala and frontal cortex which are considered critical for anxiety, depression and cognition. Sprague Dawley rats were randomly assigned in four groups; control, exercised, social defeat, social defeat and exercise. At the end of the SD or control exposure lasting 30 min daily for 7 days, one group of SD rats was subjected to treadmill exercise for 2 weeks, whereas the other SD group was handled without exercise. Anxiety-like behavior tests and radial arm water maze test suggested that moderate treadmill exercise rescued social defeat induced anxiety-like behavior and memory impairment. Moreover, exercise normalized SD-induced increase in oxidative stress, most likely by adjusting antioxidant response. Our data suggests involvement of epigenetic mechanisms including histone acetylation of H3 and modulation of methyl-CpG-binding in the hippocampus that might contribute to the rescue effects of exercise in SD-induced behavioral deficits in rats.

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Statement of Interest

None

Keywords

Social defeat; stress; treadmill; exercise; anxiety and depression; Physical exercise; Anxiety; Cognition; Oxidative stress

1. Introduction

Negative impact of chronic psychological stress on an individual's physical and social performance as well as overall quality of life is well recognized (Cohen S and Wills TA 1985). While some negative effects of stress cause acute psychological reactions such as nervous breakdown, poor concentration, irritability and sleeplessness [1], others result into a chronic prolonged state of compromised mental health often leading to serious psychiatric disorders [2]. Actually, chronic stress is believed to contribute to anxiety disorders, depression [3, 4], and cognitive impairment [5–14]. Physical exercise is considered beneficial against stress, anxiety and depression, and also known to improve executive functioning and working memory [15, 16]. In fact, regular physical exercise is proposed as a neuroprotective strategy [17–19]. While antidepressant, anxiolytic and pro-cognitive effects of physical exercise are commonly accepted [20–22], the molecular basis for beneficial effects of exercise on stress-induced anxiety, depression and learning-memory impairment and mechanisms by which physical exercise alters brain function to enable neuroprotective properties are unclear.

Two major questions were addressed in this study. *First*, whether physical exercise rescues adverse behavioral consequences of stress in an animal model of social stress (defeat)? *Second*, reveal molecular pathways including oxidative stress and epigenetic mechanisms which potentially enable rescue of socially defeated phenotype. Social conflicts in humans are known to cause severe stress leading to serious psychological problems [23, 24]. Thus researchers have often utilized an ethologically relevant animal model of social stress (resident-intruder paradigm) to understand the etiology of stressor-related illnesses [25–28]. In this model, social stress induces long-lasting, adverse physiological, behavioral and neuronal deficits, which seem to resemble certain human psychopathologies of depression and anxiety [29]. Socially defeated animals also exhibit cognitive impairment [30]. This model involves aggressive encounters by a large, aggressive male rat (resident) toward a smaller male rat (intruder) [28]. Effect of physical exercise to rescue social defeat-induced deficits, have not been examined, therefore, using the social defeat model of stress, we have investigated exercise mediated behavioral and biochemical effects in rats.

Relevant to this, oxidative stress has been implicated in the response to stress [31] and in the pathogenesis of psychiatric diseases [32]. Earlier, we have published causal role of oxidative stress in anxiety-like behavior and cognitive impairment in rats and preventive effect of moderate treadmill exercise on oxidative stress–induced anxiety-like behavior [33, 34]. Moreover, social defeat has been shown to alter brain-derived neurotrophic factor (BDNF) [25] in the hippocampus [35] and exercise is known to exert a strong influence on brain plasticity and cognition, through mechanisms centered on the action of BDNF involving epigenetic mechanisms. Indeed, recent studies have found changes in modifications at specific gene promoter regions in association with social defeat [36, 37] and exercise has

been related to chromatin remodeling, specifically the induction of histone acetylation through modulation of histone deacetylases (HDAC) and histone acetyltransferases (HAT) activities. Furthermore, increased acetylation at specific promoter regions of brain-derived neurotrophic factor has been associated with a reversal of depressive-like behavior following electroconvulsive shock therapy, while overall increased acetylation in the nucleus accumbens has been associated with depressive-like symptoms in mice [37, 38]. Finally, oxidative stress is known to regulate histone acetylation/deacetylation [39, 40] and also reported to lead to the release of pro-inflammatory cytokine interleukin-8 (IL-6) in human alveolar epithelial cells via modulation of histone acetylation/deacetylation processes [41]. With these previous studies in mind, using the social defeat model of social stress, we investigated the association between oxidative stress, inflammation, histone H3 acetylation/deacteylation, methyl-CpG-binding protein (MeCP)-2 levels and BDNF levels, in the hippocampus, the amygdala, and the frontal cortex, brain areas implicated in the symptomatology of anxiety, depression and cognition both in rodent models and humans [42–47].

2. Materials and Methods

2.1. Animals

Male Sprague Dawley rats (275–300 g) were used as controls or intruders, and male Long-Evans (LE), retired breeders (400–500 g) served as residents (Charles River, Wilmington, MA). Rats were singly housed with a 12-h light, 12-h dark cycle (lights on at 0600 h) in a climate-controlled room with food and water provided *ad libitum*. All experiments were conducted in accordance with the NIH guidelines using approved protocols from the University of Houston Animal Care Committee.

2.2. Social Defeat Model

2.2.1. Experimental design—The social defeat model used in the present study was modified from the resident-intruder model originally developed by Miczek [48]. Rats were randomly assigned to either a social defeat or control group [28, 49, 50]. This paradigm consisted of 7 encounters, carried out for 7 consecutive days, with an aggressive male Long Evans (LE) rat. Each intruder (Sprague Dawley) was defeated by six different resident LE rats. [50, 51]. A typical social defeat was observed by intruder defeat, indicated by the intruder surrendering or acquiring a supine position for approximately 3 sec. After defeat, a perforated Plexiglas partition was placed in the cage to avoid direct physical contact between the LE and intruder. The Plexiglas partition with holes allowed intense visual, auditory, and olfactory interactions for the remainder of the 30-min session. If a resident struggled to defeat the intruder for 10 min, rats were separated with the Plexiglas partition for the remainder of the 30-min session. Controls were placed behind a Plexiglas partition in a fresh cage for 30 min daily. Rats were returned to their home cage after each social defeat session, and body weight was recorded on days 1 and 8. All Sprague Dawley rats were used for behavioral assessment before sacrifice.

2.3. Moderate treadmill exercise

The rats were subjected to treadmill exercise on a motorized rodent treadmill (Columbus Instruments, Columbus, OH). The treadmills are equipped with bars that deliver a mild electric shock (0–0.5 mA with a 1–2 s inter-pulse interval) when the rat stops running. This is a very mild shock which causes no stress and minimal discomfort to the animals but serves as a cue for the rat to continue running [33]. The rats were subjected to the following treadmill exercise protocol for a total of 2 weeks: 30 min daily for 2 weeks. For the first week the animals ran at 10meters/min for 30 min, followed by 15meters/min for the second week [33]. The rats were given a rest period of 5 min after 15 min of exercise in each setting.

2.4. Tests for Anxiety-Like Behavior

2.4.1. Open Field (OF) activity—Rats were placed in the center of the OF (60×40 cm) and left free to explore the arena for 15 min and movement quantified using Opto-Varimex Micro Activity Meter v2.00 system (Optomax, Columbus Instruments; OH) as previously published by us [33, 34]. Total activity, ambulatory activity, distance covered and fecal boli were examined.

2.4.2. Light-Dark (LD) exploration—Time spent in light is considered as a measure of anxiety-like behavior. The light-dark box consisted of a light and a dark compartment separated with a single opening for passage from one compartment to the other and total time spent in the lit area was recorded [33, 34].

2.4.3. Elevated plus-maze—A standard rat elevated plus-maze with 43 cm arms extending from a 10 cm central area was obtained from Med Associates Inc., (St. Albans, VT). The arms of the maze were approximately 90 cm above the floor. The rat's movements were tracked manually. The observer was blinded to the group classification to avoid bias. Each session was started by placing the rat in the central area facing the open arms of the maze and lasted 5 min. In between rats, the maze was wiped down with alcohol. The amount of time the rat spent in the open arms was noted [52].

2.5. Memory Function

2.5.1. Radial Arm Water Maze (RAWM)—The RAWM procedures were done as previously published by us [53, 54]. Basically, the apparatus consisted of a black circular pool filled with water containing six swim paths in a dimly lit room. Each rat was randomly assigned a goal arm which contains a hidden black platform near the end of the arm. The rats were randomly released at an arm different from the goal arm, allowed to swim and locate the platform which is submerged 1 cm under water. The rats were allowed 1 minute for each learning trial or memory test. An error was counted when the rat entered more than halfway into an arm other than the goal arm or if the rat entered more than half of the goal arm but failed to approach the platform. Number of errors ranged from 1–7, as the rat can only swim into 7 arms within 1 minute. If the rat failed to locate the platform within 1 minute, the rat was manually guided to the platform and was scored with 7 errors. Upon reaching the platform, the rat was allowed 15 seconds rest before the next trial began.

2.5.2. Short-term and long-term memory tests—The rats were subjected to the first set of six learning trials (trials # 1–6) followed by a five min rest period and then another set of six learning trials (trials # 7–12) and tested for short-term memory 30 min after the end of 12th trial. The rats were subjected to learning trials (trials #1–12) as above. At the end of the 12th trial, the rats were returned to their home cages and 24 h later subjected to long-term memory test.

Brain Dissections and Preparation of Homogenates: Experimental and control rats were anesthetized using mild anesthesia (Isoflurane, #57319-479-06, Phoenix Pharmaceuticals) immediately after anxiety behavior tests. The brains were quickly removed and hippocampus, frontal cortex and amygdala were identified according to the atlas of Paxinos and Watson [55] and isolated and quickly frozen in liquid nitrogen and stored at −80°C until analysis as previously published by us [33, 56, 57].

2.6.1. Western Blotting—Equal amounts of brain tissue homogenate proteins diluted with 4X laemmli sample buffer were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and western blotting as previously published by us [34]. Primary antibody dilutions used were as follows; GLO-1 (1:200 dilution; Abcam, Cambridge, MA – Cat No: ab81461), GSR-1 (1:100 dilution), Cu/Zn SOD (1:1000 dilution; Cat No: 07-403), Mn SOD (1:1000 dilution; Cat No: 06-984), Acetyl-H3 (1:500 dilution; Cat No: 07-013), total-H3 (1:1000; Cat No: 05-499), HDAC5 (1:1000 dilution; Cat No:07-045) and MeCP-2 (1:1000 dilution; Cat No: 07-013), were from Millipore, Temecula, CA. CAMKIV (1:1000 dilution; Cat No: 4032S), p-p44/42 MAPK (1:200 dilution; Cat No: 9106S) and p44/42 MAPK (1:1000 dilution; 9107S) were from Cell Signaling technology, Danvers, MA. IL-6 (1:1000 dilution, Cat No: ARC0062) was from Invitrogen, Grand Island, NY. BDNF (1:1000 dilution; Cat No: sc-546), p-CREB (1:200 dilution; Cat No: sc-7978), t-CREB (1:1000 dilution; Cat No: sc-58) and β-actin (1:1000 dilution; Cat No: sc-47778) were from Santacruz biotechnology, Santacruz, CA. The membranes were incubated with respective antibody for 1 h, followed by incubation with an anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (1:1000), anti-rat horseradish peroxidase (HRP)-conjugated secondary antibody (1:1000) or anti-mouse HRP-linked secondary antibody (1:1000) at room temperature for 1 h. The images of immunoblots were captured by a Fluorchem 8900 imaging system with intensity of each immunoreactive band determined using Alpha Ease FC 4.0 (Alpha Innotech Corp., San Leandro, CA) that were normalized to β-actin protein loading control.

2.6. Indices of oxidative stress

8-isoprostane levels in serum and urine were measured using EIA kit (Cayman, Ann Arbor, MI). Isoprostanes are a family of eicosanoids of non-enzymatic origin produced by the random oxidation of tissue phospholipids by oxygen radicals [33]. The OxyBlot™ Protein Oxidation Detection Kit (EMD Millipore Corp. #S7150) was used for immunoblot detection of carbonyl groups introduced into proteins by oxidative reactions. Equal amount (20 μg) of protein homogenate from different brain regions (prepared as indicated above) were subjected to this kit based reaction following manufacturer's instructions, which allows detection of carbonylation of proteins in the homogenates using western blotting method.

2.7. Corticosterone measurement

Serum corticosterone levels, released in response to stress and anxiety [58] were measured using an EIA based kit (cat#500651, Cayman Chem. Co., Ann Arbor, MI) per manufacturer's instructions.

2.8. Statistical Analysis

Data are expressed as mean ± SEM. Significance was determined by one-way ANOVA and Tukey's post-hoc test (GraphPad Software, Inc. San Diego, CA). A value of $p < 0.05$ was considered significant.

3. Results

3.1. General parameters

Control groups including sedentary (CON) and exercise alone (EX) gained similar amount of weight while the SD and SD+EX animals gained less weight during the 7-day social defeat protocol [Control, EX, SD and SD+EX (gain in body weight in g/7days): 20 ± 5.5, 21.2 ± 6.5, 5.5 ± 2.5 and 8.5 ± 3.5, $F(3,36) = 9.33$, $p<0.05$] (Fig 2A). Food intake during 7-day social defeat protocol was not different between control groups including, sedentary and EX alone when compared to SD and SD+EX rats [Control, SD and SD+EX (g/rat/day): 25.3 ± 2.1, 25.4 ± 1.9, 23.3 ± 3.3 and 27 ± 1.6, $F(3,36) = 2.91$, $p<0.05$] (Fig. 2B). However, daily water intake increased in SD and SD+EX [Control, EX, SD and SD+EX (ml/rat/day): 31.4 ± 4.1, 37.4 ± 3.8, 51.8 ± 18.4 and 55.2 ± 15.4, $F(3,36) = 2.45$, $p<0.05$] rats when compared to controls (Fig. 2C).

3.2. Anxiety-like behavior tests

In light-dark test, a rat is exposed to a novel environment with protected (dark) and unprotected (light) areas. Unwillingness to explore the lit area and willingness to spend more time in the dark during a 5-min test session is indicative of high anxiety-like behaviors. Control rats spent more time (sec) in the light compartment (CON: 78.6.3 ± 16.9, EX: 97.2 ± 22.7), when compared to SD rats (41.2 ± 8.66, $F(3,36) = 3.83$, $p<0.05$). SD+EX rats spent significantly more time in the lit area (73.8 ± 17.1) as compared to SD rats (41.2 ± 8.66) (Fig. 3A). Elevated-plus maze model is based on rat's dislike for open spaces. This aversion leads to the behavior termed as thigmotaxis, which means avoidance to open areas by restricting movements to enclosed spaces or to the edges of a confined space. Increased amount of time spent in the closed arms during a 5-min session is indicative of high anxiety-like behavior. Amount of time (sec) the control rats spent in the open arms (CON: 68.6 ± 11.1, EX: 89.0 ± 9.2, $F(3,36) = 3.89$, $p<0.05$) and SD+EX (104.6 ± 23.5) was significantly higher than the SD (46.3 ± 11.2) rats (Fig 3B).

Furthermore, socially defeated rats had lower total (CON: 4064 ± 758.6, EX: 4161 ± 579.0, SD: 2927 ± 242.5 and SD+EX: 3680 ± 298.9, $F(3,36) = 6.13$, $p<0.05$) (Fig. 3C) and ambulatory activity (CON: 4072 ± 371.1, EX: 4639 ± 247.4, SD: 2705 ± 212.5 and SD+EX: 3409 ± 233.0, $F(3,36) = 5.13$, $p<0.05$) (Fig. 3D) and covered lesser distance (CON: 3824 ± 369.0, EX: 4133 ± 395.5, SD: 2503 ± 249.4 and SD+EX: 3217 ± 234.0, $F(3,36) = 6.55$, $p<0.05$) than the control and SD+EX rats (Fig. 3E). Moreover, number of fecal boli of SD

rats was significantly higher than controls and SD+EX rats (CON: 0.72 ± 0.30, EX: 1.1 ± 0.536, SD: 3.0 ± 0.462 and SD+EX: 0.83 ± 0.307, $F(3,36) = 3.53$, $p<0.05$) (Fig. 3F).

3.3. Memory function

Controls, SD and SD+EX rats on an average made comparable errors in the STM test, with each group making 0.5 ± 0.24, 0.6 ± 0.14, 0.5 ± 0.10 and 0.72 ± 0.33 errors, $F(3,36) = 0.37$, $p<0.05$ respectively. On the other hand, in the LTM, social defeat significantly increased the number of errors as compared to the control rats with control rats (CON and EX) making 0.25 ± 0.2 and EX: 0.6 ± 0.5 errors while SD rats made 2.6 ± 0.7 errors. SD+EX groups made 1.1 ± 0.3 error, $F(3,36) = 3.43$, $p<0.05$. Thus, social defeat did not significantly affect STM but the long-term memory consolidation was affected in these rats (Fig. 4 A, B).

3.4. Markers of oxidative stress and antioxidant enzymes

Protein carbonylation was measured in oxidative stress susceptible brain areas, previously reported to be important for anxiety and learning-memory function [34, 56, 59]. Protein carbonylation significantly increased in the hippocampus of SD rats as compared to the two control groups (CON and EX), while the levels were not altered in the frontal cortex and amygdala (Table. 1). Interestingly, protein carbonylation in the hippocampus of SD+EX rats was significantly lower than SD rats.

Protein expression levels of GLO-1, GSR-1, Cu-Zn SOD and Mn-SOD were normalized to the internal loading control β-actin and examined in the hippocampus, amygdala and frontal cortex. While Mn-SOD and Cu-Zn SOD protein expression levels decreased only in the hippocampus of SD rats, no change was observed in the frontal cortex and amygdala between all other groups. GLO-1 protein expression levels decreased in the hippocampus and amygdala, but not in the frontal cortex of SD rats, while the levels bounced back in SD +EX group. GSR-1 levels remained unchanged in all groups (Table. 1).

Plasma 8-isoprostane (Fig. 5) significantly increased in SD rats (SD: 41.0 ± 4.2) as compared to CON or EX rats (CON: 28.0 ± 1.8, EX: 28.6 ± 2.2), while SD+EX rats showed significantly reduced plasma 8-isoprostane levels (SD+EX: 24.0 ± 3.2) when compared to SD rats (41.0 ± 4.1). Thus two different markers of oxidative stress indicate that, socially defeated rats have higher oxidative stress than control rats and exercise alleviates this effect.

3.5. Plasma corticosterone

Plasma corticosterone levels significantly increased in SD (66.9 ± 6.4 ng/ml) rats when compared to the control rats (CON: 31.8 ± 3.1 ng/ml, EX: 30.3 ± 3.5 ng/ml), while SD+EX group exhibited significantly reduced levels (54.31 ± 0.2 ng/ml) when compared to SD rats (Fig. 6).

3.6. Molecules involved in memory consolidation and inflammatory markers

Social defeat significantly decreased the protein levels of BDNF, p-CREB/total CREB and CAMKIV in the hippocampus when compared to control rats (CON and EX). The levels of all three proteins significantly increased in the hippocampus of SD+EX rats when compared

to SD rats. The levels of these proteins remained unchanged in the frontal cortex and amygdala (Table 2).

Induction of social defeat stress caused ERK-1/2 activation (phospho ERK-1/2 normalized to total ERK-1/2 protein) in the hippocampus and amygdala but not in the frontal cortex (Table 2). SD+EX rats showed significantly lesser ERK1/2 activation when compared to SD rats. Moreover, ERK-1/2 activation was associated with upregulation of an inflammatory cytokine IL-6, in the cortex and the hippocampus of SD rats when compared to SD or EX rats (Table 2).

3.7. Histone H3 acetylation and histone deacteylase (HDAC5) levels

The protein levels of histone H3 acetylation and histone deacetylase (HDAC5) were assessed in the hippocampus, amygdala and frontal cortex of rats (Fig. 7). SD rats exhibited significantly reduced levels of H3 as compared to CON or EX rats in the hippocampus. SD +EX rats on the other hand showed significantly greater levels of H3, when compared to SD rats in the hippocampus (A–C). No significant changes were observed in the amygdala or the frontal cortex. HDAC 5 showed significantly higher levels in SD rats, when compared to CON or EX rats while the level was normalized in SD+EX group within the hippocampus. No significant changes were observed in the frontal cortex or amygdala. HDAC 5 showed significantly higher levels in SD rats when compared to CON or EX rats while the level was normalized in SD+EX group within the hippocampus (D–F). No significant changes were observed in the frontal cortex or amygdala.

3.8. Methyl-CpG-binding protein (MeCP)-2 levels

SD rats exhibited significantly reduced protein levels of MeCP-2 as compared to CON or EX rats in the hippocampus (Fig. 8). SD+EX rats on the other hand showed significantly greater levels of MeCP-2 when compared to SD rats in the hippocampus as well as in the amygdala (A–C). No significant changes were observed in the amygdala or the frontal cortex in SD rats but both hippocampus and amygdala showed marked increase in MeCP-2 levels.

4. Discussion

Our results suggest that social defeat-mediated stress increase anxiety-like behavior of rats assessed via LD, EPM and OF behavior tests. Display of increased anxiety-like behavior is indicated by reduced time spent by SD rats in the lit area of the LD box, and reduced time spent in the open-arms of the EPM apparatus. Reduced ambulatory activity, total activity and less distance travelled in the OF arena by SD rats also indicates heightened anxiety-like behavior. Greater number of fecal boli of SD rats also indicates high anxiety-like behavior. Interestingly, SD+EX rats did not show anxiety-like behavior as their anxiety levels were comparable to that of control rats, suggesting that treadmill exercise had a protective effect on anxiety-like behavior. It is possible that SD rats were able to better cope with social defeat-induced stress when subjected to treadmill exercise and their SD-induced anxious phenotype was recused with moderate treadmill exercise intervention. Previously, moderate treadmill exercise regimen used by us, also resulted in prevention of anxiety-like behavior in

sleep-deprived rats [34]. Others also have reported anxiolytic effect of treadmill as well as wheel running in rats and several human studies have indicated anxiolytic effects of exercise [60, 61]. However, some have observed either no change or have reported anxiety-inducing behavior with exercise [34, 62, 63]. The divergent results are most likely due to the different regimens of exercise used and different anxiety tests employed and different parameters assessed. Furthermore, social defeat induced long-term memory deficits observed in SD rats also were rescued with moderate treadmill exercise. Beneficial effects of physical exercise on cognitive function in humans as well as in laboratory animals [64–67] are known and impairment of learning and memory processes has been demonstrated by many studies using different stressors [68]. Exposure to chronic restraint stress in rats and psychosocial stress in humans is known to alter cognitive functions and also has been linked to the pathophysiology of many disorders [69]. While protective effect of exercise on stress-induced deficits are convincing, reports that exercise does not have significant protective effects on memory deficit in stressed rats also exist [70, 71].

Overall, our data is in agreement with previous reports which support that exercise improves anxiety [72, 73], depression [74–78], cognitive function [79–83] and overall mental well-being [84, 85].

Consistent with previous findings [28], socially defeated rats did not attain normal rodent weight gain profile and exercise did not contribute to any weight gain in the SD+EX group. All rats consumed comparable amount of diet, but SD and SD+EX rats drank more water. There is no clear consensus on the effect of exercise on body weight gain and food intake, with some studies reporting that exercise promotes reduced body weight gain and decreased food intake [86, 87], while others suggesting no effect of exercise on body weight but report increased food intake as a consequence of exercise [88]. Our studies fit well with that of Applegate et al (1982) and others [86, 87, 89].

Elevated stress indicated by increased plasma corticosterone levels in socially defeated rats was markedly reduced in SD+EX rats, suggesting protective effect of treadmill exercise on SD-induced stress. Earlier, protective effect of treadmill exercise on increased corticosterone levels in sleep-deprived rats has been reported [34]. Furthermore, role of oxidative stress in the protective effects of treadmill exercise is also known [33, 34]. Moderate exercise is known to cause adaptation of brain antioxidant system by increasing its resistance to oxidative stress [33, 90, 91], while exhaustive exercise is reported to enhance lipid peroxidation [56, 92, 93] and known to increase reactive oxygen species, leading to oxidative damage [94–96]. Relevant to this, we observed that increase in oxidative stress in SD rats was reversed when SD rats were subjected to treadmill exercise. The decline in antioxidant enzyme expression including GLO-1 in hippocampus and amygdala and GSR-1, Mn-SOD and Cu/Zn SOD in the hippocampus only, was normalized with exercise. This is in agreement with our recent reports where modulation of these antioxidant enzymes has been observed in specific brain areas including the hippocampus and the amygdala [97–99]. Increased oxidative stress is a result of reduced antioxidant response which most likely occurs due to diminished GLO-1, GSR-1, Mn SOD and Cu/Zn SOD protein expression. It seems reasonable to suggest that reduced levels of these antioxidant enzymes contribute to a failing antioxidant response which leads to an excessive accumulation of reactive oxygen/

nitrogen species, leading to inflammation and cytotoxicity. This is in keeping with increased expression of IL-6 and mitogen-activated protein kinase ERK-1/2 observed in the hippocampus and cortex of SD rats. Reduced levels were normalized with exercise treatment. ERK-1/2–mediated increase in inflammatory markers is well known [100]. Exercise also normalized social defeat-induced decrease in the levels of CAMKIV, p-CREB/total CREB and BDNF protein levels in the hippocampus but not in the amygdala or the cortex. These observations are quite significant considering that social defeat has been shown to alter brain-derived neurotrophic factor (BDNF) [25] in the hippocampus [35] and exercise is known to exert a strong influence on brain plasticity and cognition, through epigenetic mechanisms centered on BDNF. Interestingly, treadmill exercise normalized SD-induced decreased histone H3 acetylation and also normalized SD-induced increase in HDAC5 protein levels in the hippocampus only. Furthermore, exercise also normalized SD-induced decreased MeCP2 protein levels in the hippocampus.

5. Conclusions

Present study demonstrates that social defeat stress-induced behavioral and cognitive impairments are rescued by moderate treadmill exercise. Additionally, moderate treadmill exercise activates a pathway which involves suppression of oxidative stress and inflammation. This is enabled perhaps viamodulation of histone H3 acetylation/deacetylation processes, as well as regulation of MeCP-2 and BDNF levels. SiRNA approaches will test the causality of each component in future studies.

Acknowledgments

Funding for this research was provided to S.S. by NIH 1R15MH0939-18-01A1.

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Research Highlights

- Social defeat stress-induced behavioral and cognitive impairments are rescued by moderate treadmill exercise.
- Moderate treadmill exercise suppresses social defeat-induced activation of oxidative stress and inflammation.
- Moderate treadmill via histone H3 acetylation/deacetylation dependent MeCP-2 activation regulates BDNF expression.

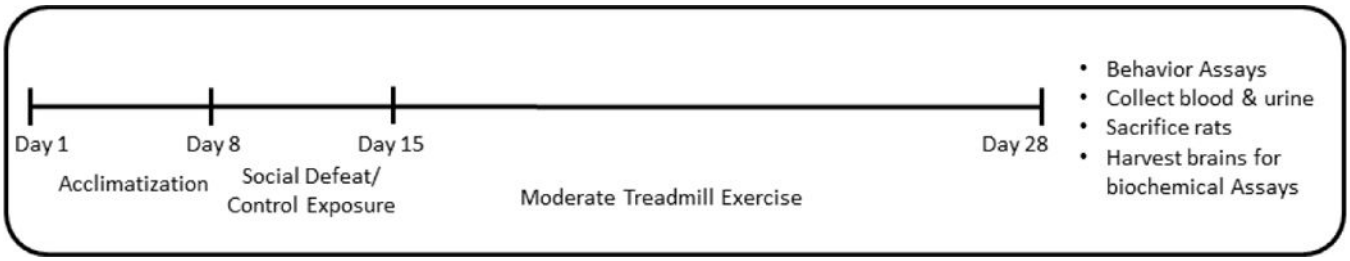

Fig. 1. Schematic representation of the experimental plan

Sprague Dawley rats were assigned into four groups; Group 1: control, group 2: exercised, group 3: social defeat; group 4: social defeat and exercise. At the end of the social defeat or control exposure, one group of socially defeated rats was subjected to treadmill exercise for 2 weeks (1st week – 10m/min for 30 minutes, 2nd week – 15m/min for 30 minutes) whereas the other socially defeated group was handled without the exercise).

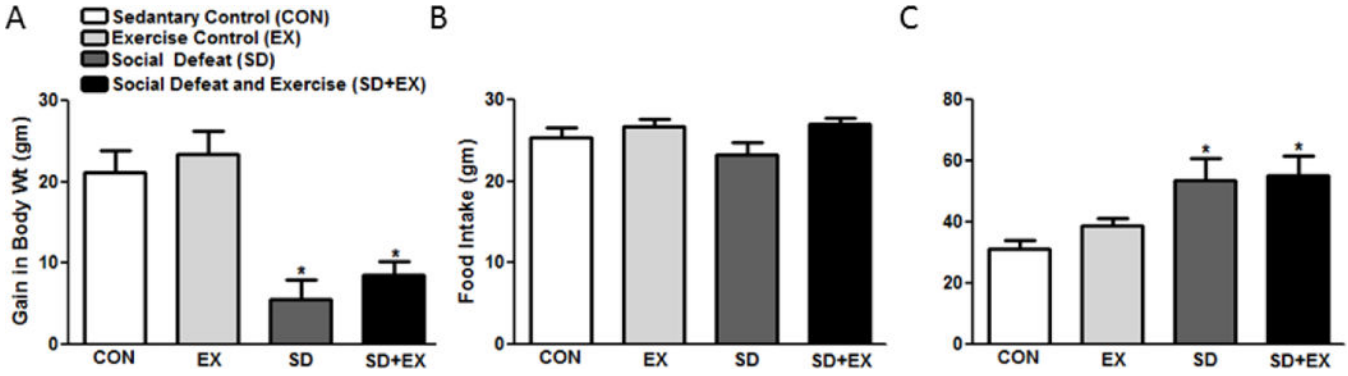

Fig. 2. General body parameters

Examination of general parameters including gain in body weight (A), food (B) and water (C) intake was measured. Four groups of male Sprague-Dawley rats were utilized in this study. Group 1: control (sedentary), group 2: exercised, group 3: social defeat; group 4: social defeat and exercise. Bars are means ± SEM, n = 10 rats/group. *significantly different from control (sedentary and exercise alone) rats, #significantly different from SD (social defeat) rats, $p<0.05$.

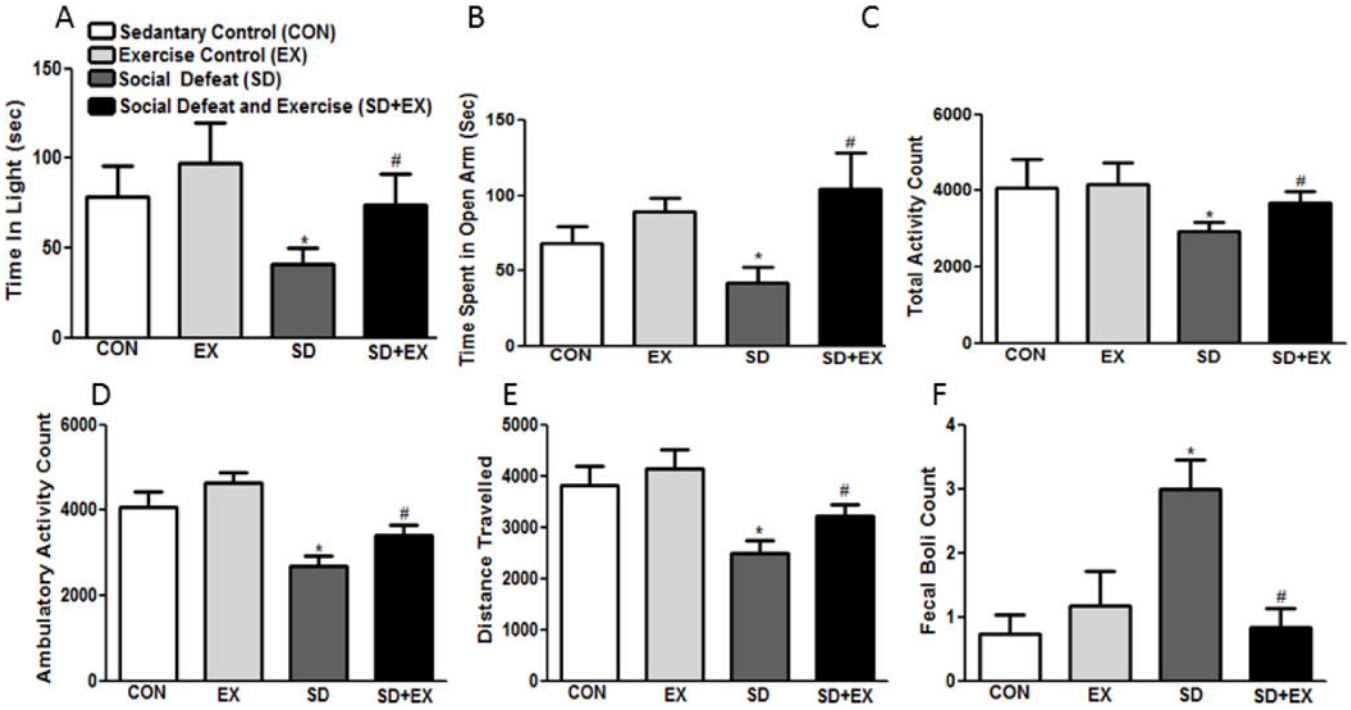

Fig. 3. Examination of anxiety-like behavior using light-dark, elevated-plus maze and open-field tests

Light-dark test determined time spent in light (A), elevated-plus maze test determined time spent in open arms (B) while the open-field test determined total activity (C), ambulatory activity (D), distance traveled (E) and fecal boli (D). Bars are means ± SEM, n = 10 rats/group. *significantly different from control (sedentary and exercise alone) rats ($p<0.05$), #significantly different from SD rats ($p<0.05$) using one way ANOVA analysis.

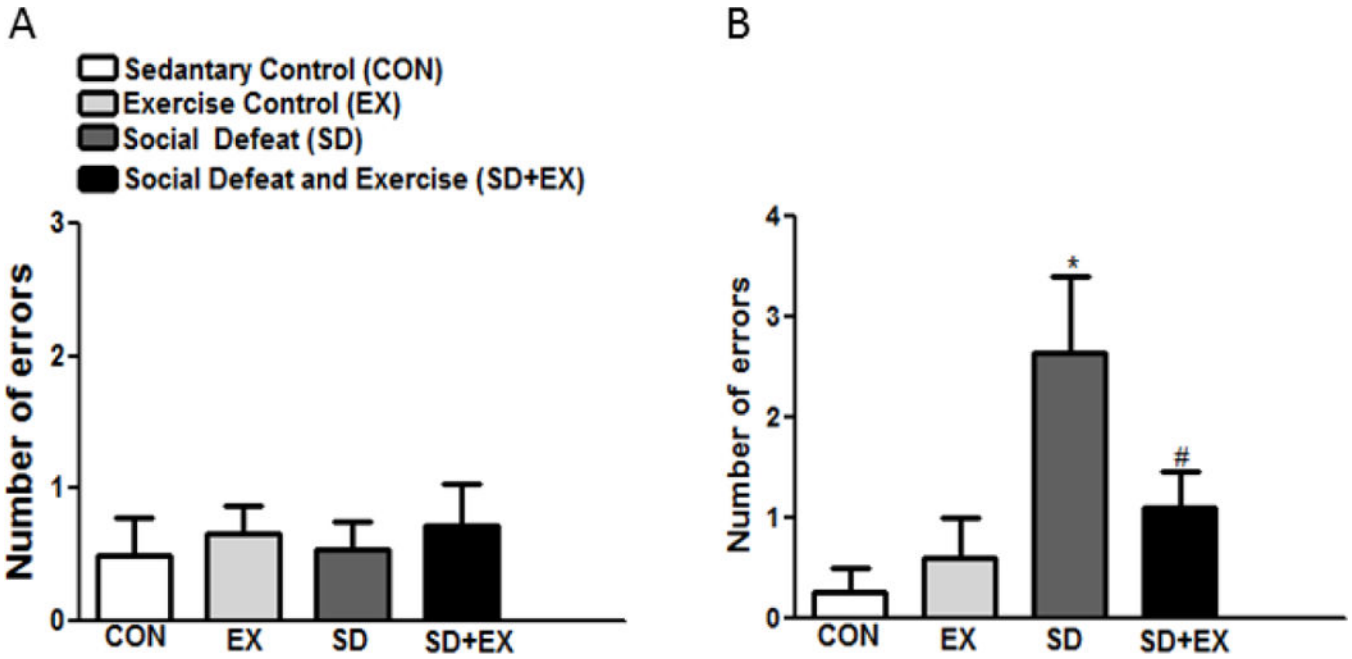

Fig. 4. Examination of short-term and long term memory using radial arm water maze (RAWM) memory test

Short term (A) and long term (B) memory was assessed using a series of twelve RAWM trials. Bars are means ± SEM, n = 10 rats/group. *significantly different from control (sedentary and exercise alone) rats ($p<0.05$), #significantly different from SD rats ($p<0.05$) using one way ANOVA analysis.

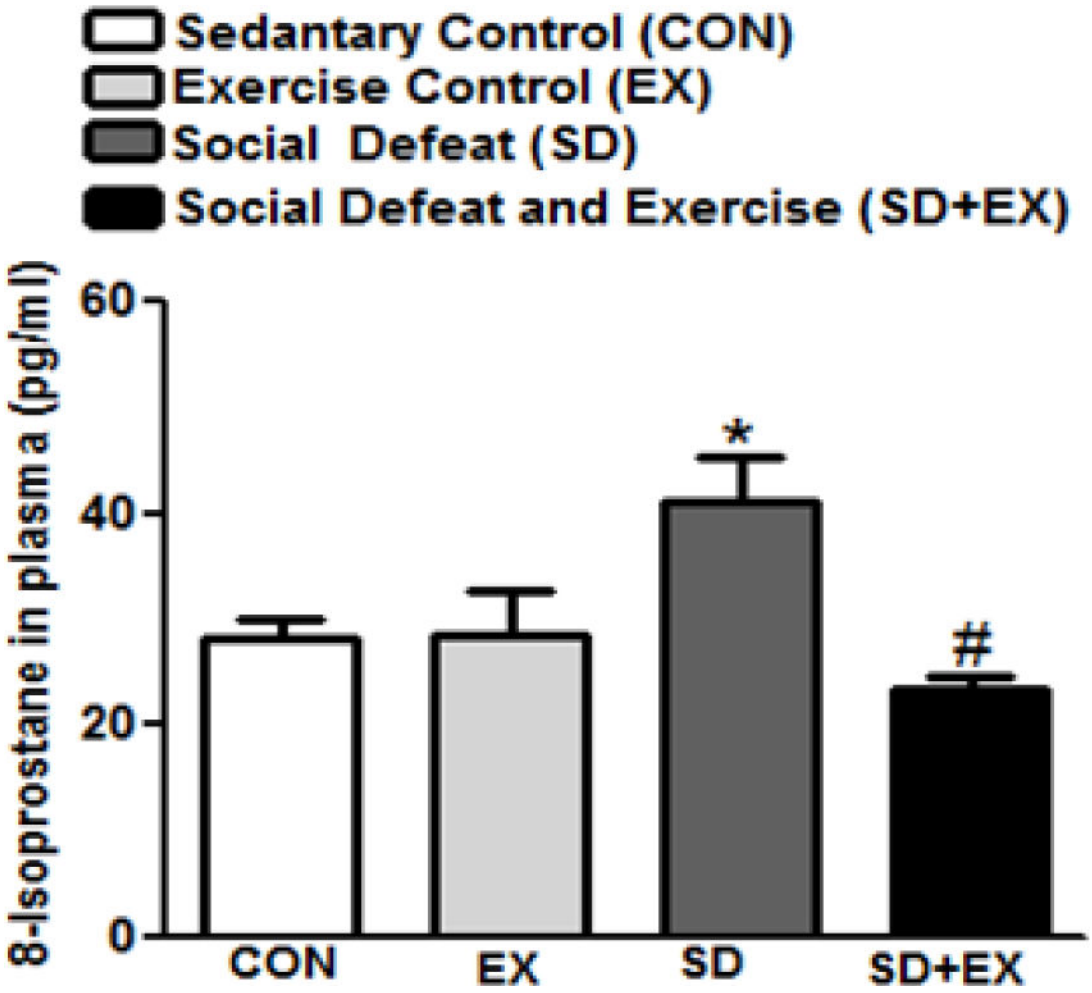

Fig. 5. Analysis of 8-isoprostane in plasma of rats

8-isoprostane was measured using EIA kit (516351; Cayman, Ann Arbor, MI). *significantly different from control (sedentary and exercise alone) rats ($p<0.05$), #significantly different from SD rats ($p<0.05$) using one way ANOVA analysis, n = 6–10 rats/group.

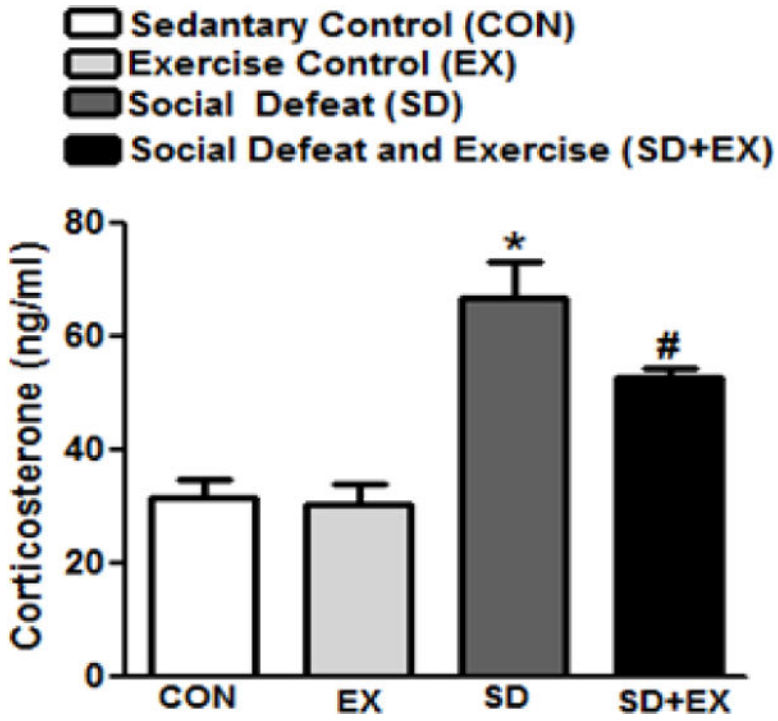

Fig. 6. Analysis of corticosterone levels in plasma of rats

Plasma corticosterone, released in response to stress was measured using an EIA based kit (cat#500651, Cayman Chem. Co., Ann Arbor, MI) per manufacturer's instructions. *significantly different from control (sedentary and exercise alone) rats ($p<0.05$), #significantly different from SD rats ($p<0.05$) using one way ANOVA analysis, n = 10 rats/group.

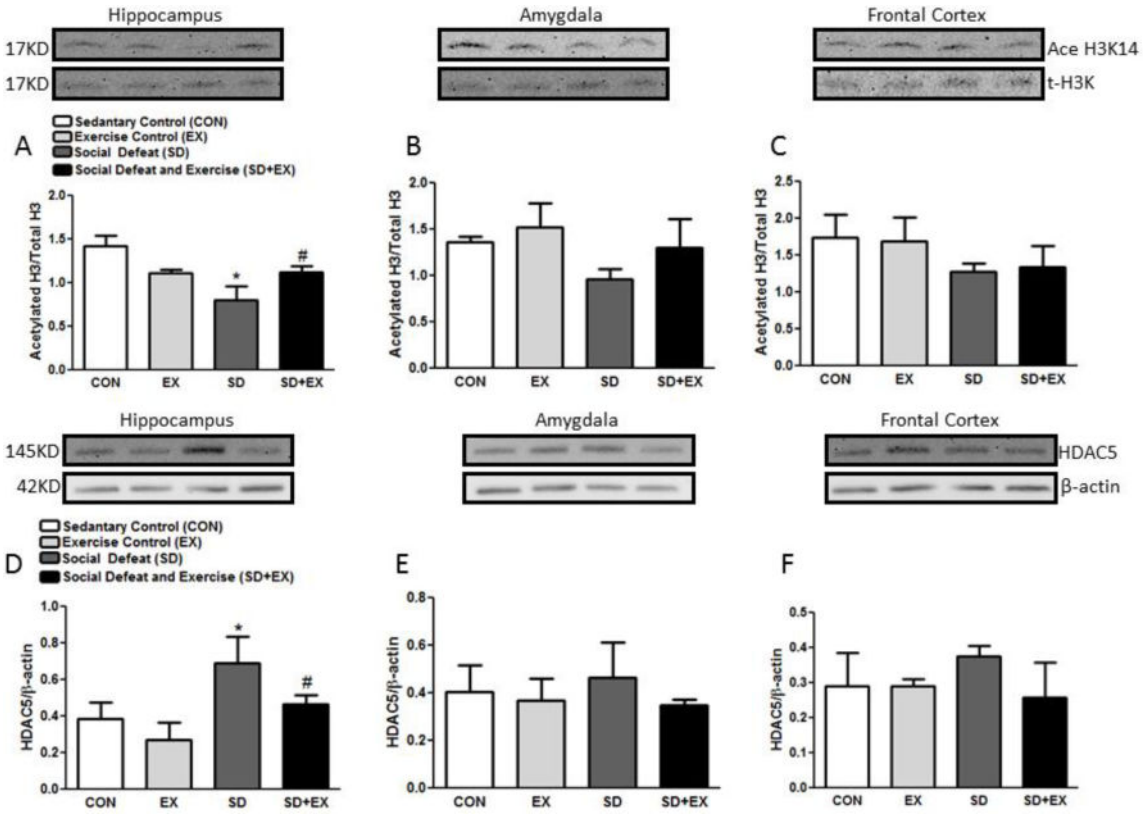

Fig. 7. Analysis of proteins involved in histone acetylation and deacetylation

Protein levels of acetyl-H3/t-H3 and HDAC5/β-Actin were determined by Western blotting in the hippocampus, amygdala and the frontal cortex. Protein ratios were obtained by normalizing to loading control/total protein as indicated. *significantly different from control (sedentary and exercise alone) rats ($p<0.05$), #significantly different from SD rats ($p<0.05$) using one way ANOVA analysis, n = 4–6 rats/group.

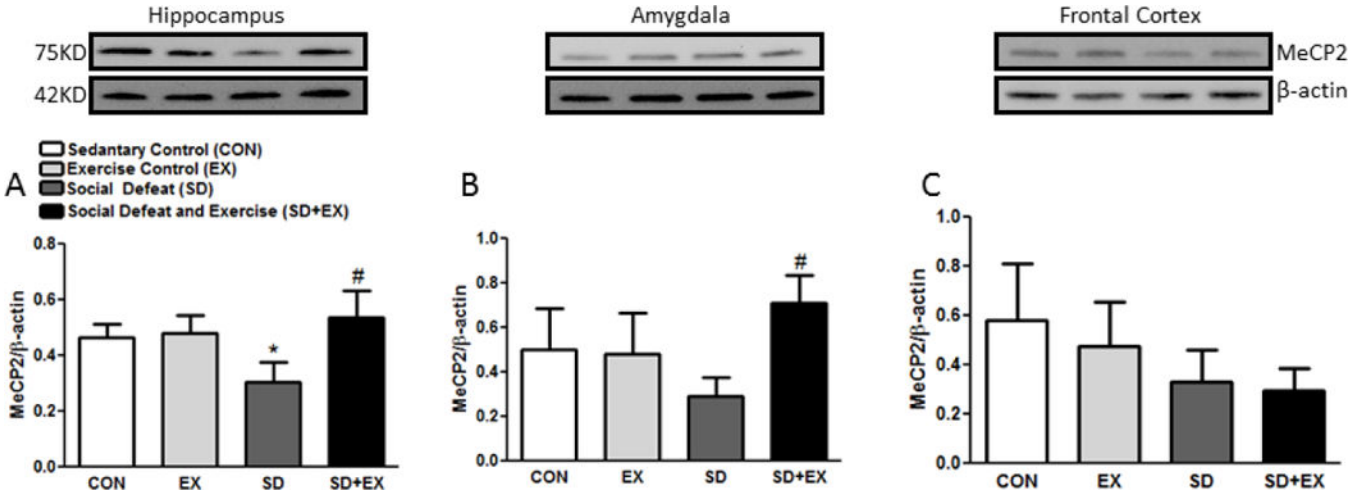

Fig. 8. The level of methyl-CpG-binding protein (MeCP)-2

The protein level of MeCP-2/β-Actin was measured in the hippocampus, amygdala and frontal cortex of rats by Western blotting (A–C). Protein ratios were obtained by normalizing to loading control β-Actin as indicated. *significantly different from CON and EX rats, #significantly different from SD rats ($p<0.05$) one way ANOVA analysis, n = 4–6 rats/group.

Table 1

Analysis of oxidative stress markers/antioxidant enzymes, proteins involved in neuroprotection and inflammation.

Markers of Oxidative Stress/ Antioxidant Enzymes	Hippocampus				Amygdala				Frontal Cortex			
	Con	Ex	SD	SD + Ex	Con	Ex	SD	SD + Ex	Con	Ex	SD	SD + Ex
Prate in Carbonylation (A.U. × 10^3)	92.08±4.1	90.0±4.1	185.88±14.8*	102.2±12.8#	102.0±4.08	109.0±4.12	137.81±14.0*	100.2±12.8#	192.0±14.3	190.0±12.1	195.88±12.7	189.2±10.8
GLO-1/β-actin	1.08±0.09	1.11±0.02	0.76±0.08*	1.18±0.1#	0.98±0.04	1.06±0.1	0.76±0.05*	0.88±0.04#	0.75±0.01	0.88±0.03	0.75±0.06	0.66±0.04
GSR-1/β-actin	2.21±0.13	1.98±0.02	1.61±0.11*	1.93±0.13#	1.91±0.07	1.98±0.02	1.91±0.11	2.1±0.71	1.81±0.13	1.66±0.02	1.59±0.11	1.79±0.13
Mn-SOD/β-actin	0.77±0.05	0.68±0.07	0.27±0.02*	0.65±0.01#	0.82±0.05	0.88±0.05	0.83±0.02	0.87±0.01	0.55±0.01	0.43±0.01	0.39±0.03	0.44±0.06
Cu-Zn SOD/β-actin	0.52±0.09	0.55±0.03	0.33±0.04*	0.45±0.03#	0.62±0.12	0.67±0.12	0.53±0.04	0.63±0.04	0.45±0.08	0.44±0.06	0.28±0.08	0.56±0.08

Protein carbonylation was measured in the hippocampus, amygdala and the frontal cortex using the OxyBlot™ Protein Oxidation Detection Kit following manufacturer's instructions. Protein levels of GLO-1/β-Actin, GSR-1/β-Actin, Cu-Zn SOD/β-Actin and Mn-SOD/β-actin were determined by Western blotting in the hippocampus, amygdala and the frontal cortex. Protein ratios were obtained by normalizing to loading control protein as indicated. Group 1: control (sedentary, CON), group 2: exercised (EX), group 3: social defeat (SD); group 4: social defeat and exercise (SD+EX).

*significantly different from control (sedentary and exercise alone) rats ($p<0.05$),

#significantly different from SD rats ($p<0.05$) using one way ANOVA analysis, n = 4–6 rats/group.

Physiol Behav. Author manuscript; available in PMC 2015 May 10.

Table 2

Analysis of proteins involved in neuroprotection and inflammation.

Molecules involved in Memory consolidation	Hippocampus				Amygdala				Frontal Cortex			
	Con	Ex	SD	SD + Ex	Con	Ex	SD	SD + Ex	Con	Ex	SD	SD + Ex
BDNF/β-actin	1.25+0.03	1.37±0.01	0.55±0.04*	1.07+0.01#	1.31±0.02	1.40±0.04	0.91±0.02	1.10±0.07	1.28±0.01	1.31±0.02	1.22±0.02	0.97±0.07
p-CREB/t-CREB	0.48±0.05	0.43±0.03	0.22±0.01*	0.38±0.03#	0.21±0.03	0.33±0.01	0.16±0.01	0.14±0.02	0.34±0.03	0.40±0.05	0.31±.05	0.30±.01
CAMKIV/β-actin	0.55±0.02	0.53±0.02	0.20±0.03*	0.41±0.02#	0.42±0.02	0.43±0.04*	0.36±0.07	0.34±0.02	0.51±0.06	0.60±0.04	0.53±.03	0.50±.02
Inflammatory Markers												
p-ERK[1/2)/t-ERK(1/2)	0.23+0.04	0.20±0.03	0.57±0.04*	0.38±0.01#	0.20±0.02	0.22+0.03	0.60±0.03*	0.41±0.01#	0.33±0.02	0.30±0.03	0.37±0.02	0.4±0.01
IL-6/β-actin	0.44+0.01	0.42±0.02	0.80±0.01*	0.58±0.04#	0.50±0.01	0.42±0.02	0.60±0.01	0.58±0.04	0.54±0.02	0.42±0.02	0.90±0.03*	0.68±0.03#

Protein levels of BDNF/β-Actin, p-CREB/t-CREB, CAMKIV/β-Actin, p-ERK1/2/t-ERK1/2 and IL-6/β-Actin were determined by Western blotting in the hippocampus, amygdala and the frontal cortex. Protein ratios were obtained by normalizing to loading control/total protein as indicated. Group 1: control (sedentary, CON), group 2: exercised (EX), group 3: social defeat (SD); group 4: social defeat and exercise (SD+EX).

* significantly different from control (sedentary and exercise alone) rats ($p<0.05$),

significantly different from SD rats ($p<0.05$) using one way ANOVA analysis, n = 4–6 rats/group.

Physiol Behav. Author manuscript; available in PMC 2015 May 10.