Original Article

Regular Exercise Training Enhances Spatial Memory and Regulates Glucocorticoid System in Experimental Autoimmune Encephalomyelitis

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Abstract

Background & objective: Exercise has been shown to improve cognitive function in patients with multiple sclerosis (MS). Experimentally, glucocorticoids (GCs) treatment has been observed to improve cognitive deterioration in an autoimmune model for MS, experimental autoimmune encephalomyelitis (EAE). We aimed to determine the combined effect of exercise and 4 mg/kg of dexamethasone (Dex) for 4 weeks on spatial memory in EAE.

Materials & Methods: Rats with EAE were subjected to the Morris water maze (MWM) for four days and a prop test for one day. The prop test was repeated on day 40 post-induction (dpi). Rats were randomly assigned to one of four groups (10 rats per group): control EAE without treatment; EAE + dexamethasone, (EAE + Dex); EAE + exercise (EAE + Ex); and EAE+Dex+Ex. Rats receiving dexamethasone were administered 4 mg/kg injections daily for two weeks after EAE induction. Exercise training was initiated on a motorized treadmill 2 weeks before EAE induction and continued until 14 dpi. On day 41, animals were dissected and CORT level was assessed by enzyme-linked immunosorbent assay corticosterone kit.

Results: One-way analysis of variance (ANOVA) with repeated measures followed by a protected LSD post hoc test indicated that, EAE+Ex group increased body weight ($P < 0.001$) and it displayed a significantly lower CORT concentration ($P < 0.001$) with delayed clinical score until day 13 dpi. Further EAE+Ex improved memory by time spent ($p > 0.001$) and swimming speed ($p > 0.002$).

Conclusion: The protocol selected in this study was an effective treatment for the EAE model to improve spatial memory and regulate corticosterone concentrations.

Keywords: Regular Exercise, Experimental Autoimmune Encephalomyelitis, Dexamethasone, Memory, Corticosterone

Introduction

Experimental autoimmune encephalomyelitis (EAE) is the most ordinarily used experimental model for the human demyelinating disease of the central nervous system. EAE is a complex condition in which the interaction between a variety of immunopathological and neuropathological mechanisms leads to an approximation of the key pathological features of Multiple sclerosis (MS) (1). MS is an inflammatory demyelinating disease characterized by neurological disability and cognitive impairment. Although the precise pathomechanism of MS is not well understood, MS is known to be an immune-mediated disease in which autoimmune T cells have been proposed to play a key role (1). In patients with MS,

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cognitive dysfunction is a complex, yet frequently, reported disorder (2) ,with approximately half of all MS patients suffering from deficits in learning and memory (3). Pharmacological interventions for cognitive impairment in MS can be divided into two types: (i) disease-modifying therapies (DMTs) that alter the course of the disease and may therefore have consequences for cognition; and (ii) symptomatic treatments that specifically target cognitive symptoms (4). Glucocorticoids (GCs) have also been used in MS for the management of acute exacerbations because of their anti-inflammatory function (5). It has been reported that GCs, such as methylprednisolone and dexamethasone (DEX), improve the murine model of MS, experimental autoimmune encephalomyelitis (EAE) in Lewis rats while increasing T cell apoptosis in the CNS (6, 7). The use of DEX in MS reduces the expression of major histocompatibility complex class II on microglia, which may interfere with antigen presentation and T cell activation (8). Dex is among the most commonly used GCs in MS and has been reported to be a beneficial effect in the EAE model on clinical, histological, and immunological signs of disease (9).

Exercise (Ex) training has been reported to protect against the cognitive impairments associated with MS (10, 11) and EAE (12, 13). Cassilhas and colleagues demonstrated that exercise training could enhance spatial learning and memory both in humans(14), and animals (15).They proposed that spatial discrimination improvement was due to exercise-induced neurogenesis (16). Additionally, exercise may prevent cellular and molecular deterioration of the hippocampus in the EAE model (12).

Given the large utilization of GCs in the treatment armament of MS, the study of adjuvant exercise in combination with GCs treatment is warranted.

Studying the association between exercise training and GCs therapy may provide useful insight regarding the impact on the MS pathophysiology while also providing insights into its impact on memory and learning. To our knowledge, there is no study investigating the effect of exercise training along with GCs treatment on EAE diseases after recovery from clinical disability. Here, we investigate the impacts of exercise with GCs treatment on spatial memory performance using the EAE model. We hypothesized that exercise can counteract the deleterious effects of GC therapy leading to improved memory.

Materials & Methods

Forty female Lewis rats between 6-7 weeks of age (100-125 g) from Animal Center Laboratory of Kermanshah University of Medical Sciences, Iran). The rats were housed in an environment with controlled temperature (24 ± 2 °C) and humidity (60%) with 12-h light/dark cycles, and free access to food and water ad libitum. Experiments were performed according to the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, National Research Council, Washington, DC, National Academy Press, no. 85-23, revised 1996), and animal experiments were performed according to Animal Ethics Committee of Kermanshah University of Medical Sciences (ethical code: 1398.146) and followed the international rules for animal research. EAE was induced and rats were subsequently randomized to one of four groups (10 rats per group): 1) EAE, 2) EAE + Ex, 3) EAE + Dex, and 4) EAE + Dex + Ex. The EAE group served as the study control group. The EAE + Ex group performed the treadmill exercise described below. The EAE + Dex group was injected daily with 4 mg/kg of Dex administration as described below. The EAE + Dex + Ex group received daily subcutaneous injections with 4 mg/kg of Dex administration in addition to completing the exercise protocol.

EAE induction and monitoring

EAE was induced, as previously described (9). Briefly, we injected rats with an inoculum containing 50 ug guinea pig spinal cord homogenate emulsified in incomplete Freund's adjuvant (Sigma Aldrich, St. Louis, Missouri, United States) and 2mg of heat-mycobacterium (Difco H37Ra). EAE induction was performed

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by injecting 50 ul of the emulsion subcutaneously at the tail base. Signs of disability were monitored daily using EAE disease scores and body weight changes. The severity of EAE was scored as follows: 0 no clinical signs; 1-tail paralysis; 2 hind limb paresis; 3 both hind limbs paralysis: 4-forelimbs paralysis; 5- moribund or deceased (2).

Exercise Training Protocol

Treadmill exercise has been suggested to be more efficient than voluntary exercise in neuroprotection by changing brain metabolism (17, 18). Therefore, this modality of exercise was chosen for this study. Rats were habituated to treadmill running by running on the treadmill for 15 minutes a day for seven consecutive days (belt speed, 5-10 m/min, with no slop). A low level of electric shock (0.3 mA) was used to motivate rats to run continuously.

The exercise training protocol was initiated 2 weeks before EAE induction and continued for 2 weeks after EAE induction. In the two exercise groups (EAE +Ex and EAE + Dex + Ex), the rats ran on the treadmill for 30 min/day, 5 days per week for a total of 4 weeks (12). The intensity began at a speed of 17 m/min and was increased incrementally 1 meter per min for each subsequent week. Rats in the non-exercise groups were placed on the treadmill track with (12).

Morris water maze tests (MWM)

MWM is an effective test for spatial learning and memory skills (19).The water maze tank was a black circular pool (136 cm diameter × 60 cm height) that was filled with water (22 ± 1 °C) at a depth of 25 cm. The MWM was divided geographically into four quadrants (north, east, south, and west), which were located around the circumference of the tank. A circular platform was situated in the north-west quadrant submerged approximately 1.5 cm below the surface of the water. Each trial was recorded by a video camera linked to the monitoring scheme (EthoVision XT, Tehran, Iran) which was mounted above the pool. A tracking system was utilized to evaluate the time spent and the swimming speed until the rat reached the platform.

Rats were given a two-day time to habituate to the apparatus. The experiment consisted of four consecutive days of learning and one prop day by hidden platform detecting day. Starting points were varied in a quasi-random fashion so that in each trial the subject started from each location once and never started from the same place on any day. Each rat was positioned in the water facing the wall of the pool at one of the four-set and allowed to swim to find invisible platform unobserved probe in the pool. During each experiment, every rat was given up to 60 seconds (s) to find the hidden platform.

Rats were generally guided to the platform by the investigator. If the rat reaches the hidden platform it is allowed to stay in place for 30 s. If the rat was unable to find the platform circular within 60s, it would be put on it for the 30s during the first four days. On day 5, MWT was done in which the platform was out of the rat's reach. We repeated the MWT with a single trial without the platform on day 40 dpi.

Dexamethasone Administration

Dex (Soldesam, Laboratorio Farmacologico Milanese, Milano, Italy) was dissolved in saline and a dose of 4 mg/kg was administered subcutaneously to rats randomized to receive Dex as previously described (20). All other animals were treated with the equivalent volumes of normal saline. Daily Dex treatment continued until 15 days post-EAE inoculation.

Euthanasia and Blood Collection

After the prop test on day 40 post-immunization, rats were anesthetized with ketamine/xylazine (3/1). Blood samples were collected via cardiac puncture and samples were allowed to clot for 15 min then centrifuged at 3,000 rpm for 10 min. Serum was aliquoted and stored at −80°c until use. Serum corticosterone was measured using a commercially available enzyme-linked immunosorbent assay corticosterone kit (GmbH, Germany) following the instructions of the kit manufacturer.

Statistical analysis

IBM SPSS 22.0 was used for all statistical analyses. The data are expressed as the mean ± standard error of the mean. Data were analyzed using a one-way analysis of variance (ANOVA) with repeated measures followed by a protected LSD post hoc test. Significance level was set at p> 0.05.

Results

Dexamethasone significantly reduced body weight

The comparison of groups was before EAE induction to post of EAE induction. Results showed reduced body weight post EAE induction compared to prior induction in the EAE group ($P < 0.05$). Significant bodyweight reduction was observed in the EAE+Dex group post-induction ($P< 0.001$) compared to prior induction. On the other hand, a significant increase in body weight after EAE induction was observed in rats of the EAE+Ex group ($P < 0.001$) compared to prior induction. EAE+Ex+Dex showed significant of body reduces post induction ($P < 0.001$) compared to prior induction (Chart 1).

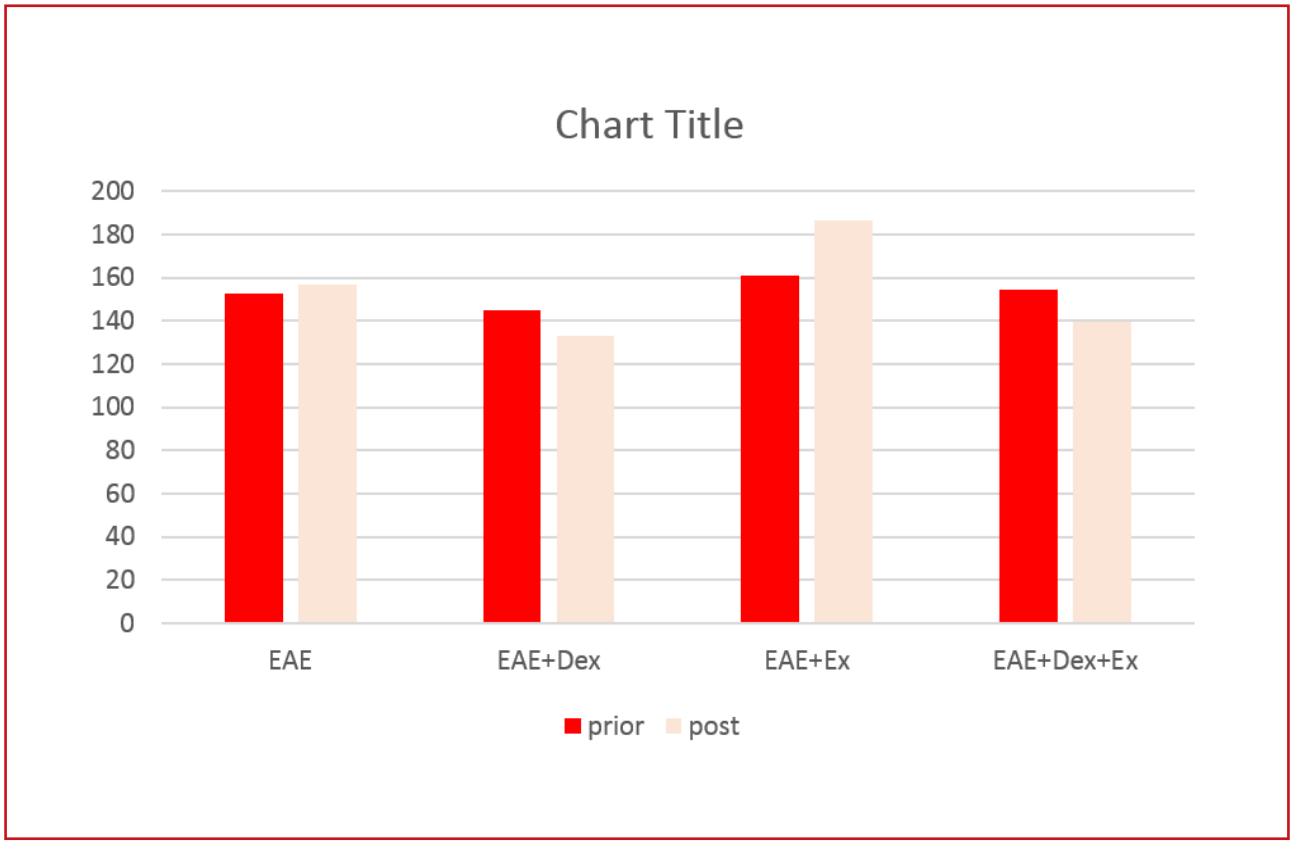

Chart 1. Body weight before (Pre) and after (Post) 40 days of intervention for four groups: EAE, EAE + Dex, EAE +Ex, and EAE + Dex + Ex. There was a statistically significant difference between Pre and Post induction, respectively. Data are presented as means ± SD

Dexamethasone delays disability

In this study, preliminary clinical signs of the EAE group appeared on day 12 post immunizations, whereas the in-group of EX showed a significant difference and delayed the onset of a clinical score to 13 dpi. In contrast, rats treated with DEX without any exercise did not show clinical signs until 21-22 dpi and readily recovery from clinical disability. However, the EX+DEX group showed clinical disability on day 22-23 dpi (Chart 2).

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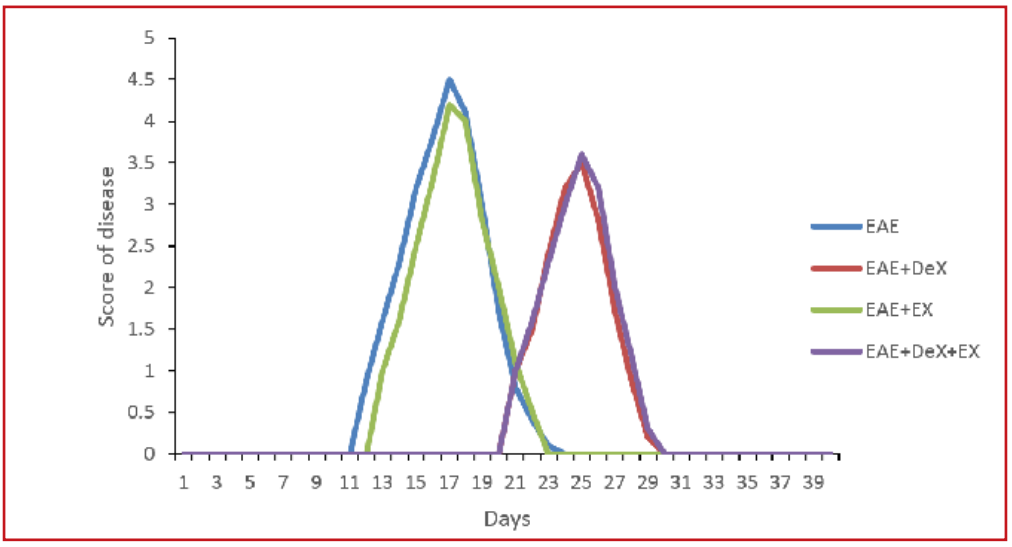

Chart 2. schematic timeline showed dexamethasone alone or with exercise delays the onset of clinical till 21 dpi. Moreover, exercise training showed delays of clinical appearance until 13 dpi. However, the EAE group began to present the motor deficits at the 12 dpi. The results are shown as mean±SEM of the experimental groups

Exercise improves spatial learning and memory in Dex treated EAE rats

Although all groups showed memory defects after EAE induction compared to prior induction, some compression among groups are shown.

To assess spatial learning and memory, the time spent in the selected quadrant was evaluated. Results showed a significant interaction among groups, as presented in Chart 3. Time spent was significantly higher in the EAE+ Ex group ($p > 0.001$) compared to the EAE group. No significant difference was observed between the EAE group and the Dex group. However, the group of EAE+Dex+Ex, showed improvement in time spent ($p > 0.05$) compared to the EAE+Dex group. No significant differences were revealed in the EAE+Dex+Ex group compared to the EAE+Ex group.

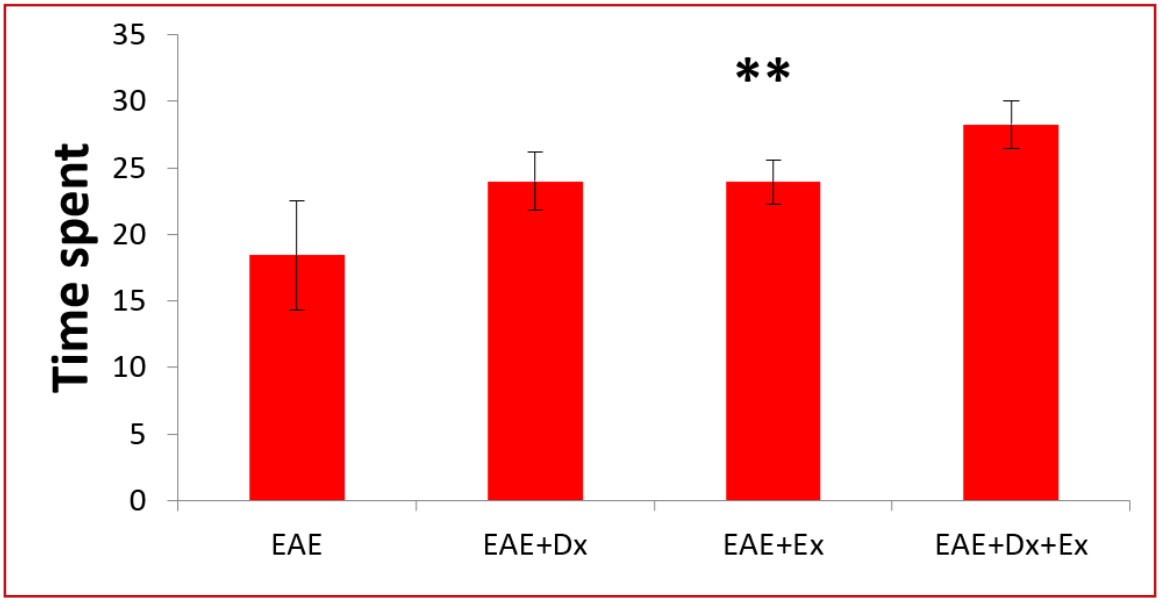

Chart 3. Time spent before (Pre) and after (Post) 40 days of intervention for four groups: EAE, EAE + Dex, EAE +Ex, and EAE + Dex + Ex was significantly different $p > 0.05$. Data are presented as means ± SD

In addition, Chart 4 shows a comparison between groups showing that swimming speed was significantly faster in the EAE+ Ex group (p> 0.02) compared to the EAE group and swimming speed, which did not show significant between EAE group compared to EAE+Dex group. An addition, it was significantly faster in EAE+ Dex+ Ex group (p> 0.001) compared to EAE + Dex and there was a slightly significant difference in swimming speed between EAE+ Ex (p> 0.02) compared to EAE+ Dex+ Ex groups.

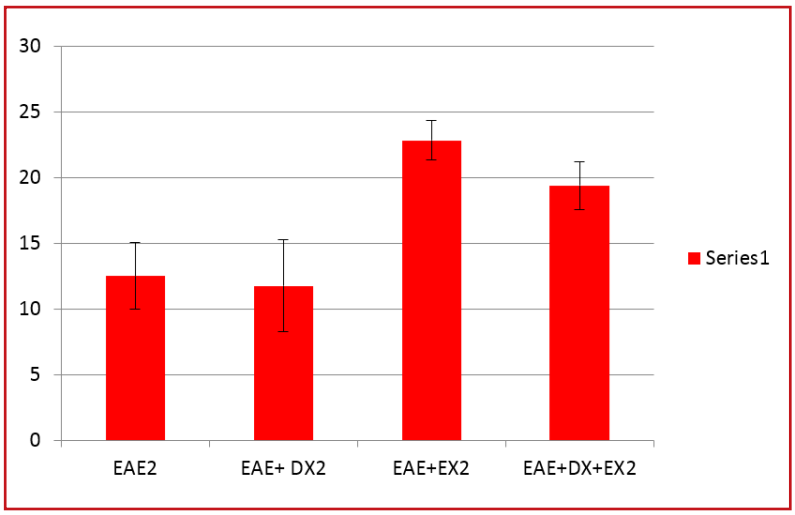

Chart 4. Swimming speed before (Pre) and after (Post) 40 days of intervention for four groups: EAE, EAE + Dex, EAE +Ex, and EAE + Dex + Ex. was significantly different p <05, p > 0.01, p > 0.001; respectively. Data are presented as means ± SD

Serum Corticosterone concentration

The results of CORT measurements are presented in Chart 5. Blood samples were collected at 8: AM-10 AM after 24h, and the significant level was considered at (P < 0.05). One-way ANOVA test revealed significant difference (F7, 31 = 7.838; P = 0.00). EAE+Ex group displayed a significantly lower CORT concentration (P <0.001) compared to the EAE group. However, a high CORT concentration level was observed in Dex groups (P>0.06) compared to the EAE group. In addition, the EAE+Ex group showed a lower level of CORT concentration (P>0.001) compared to the group of EAE+Dex+Ex. However, the EAE+Dex+Ex group showed a low-level of CORT (p>0.002) compared to the Dex group.

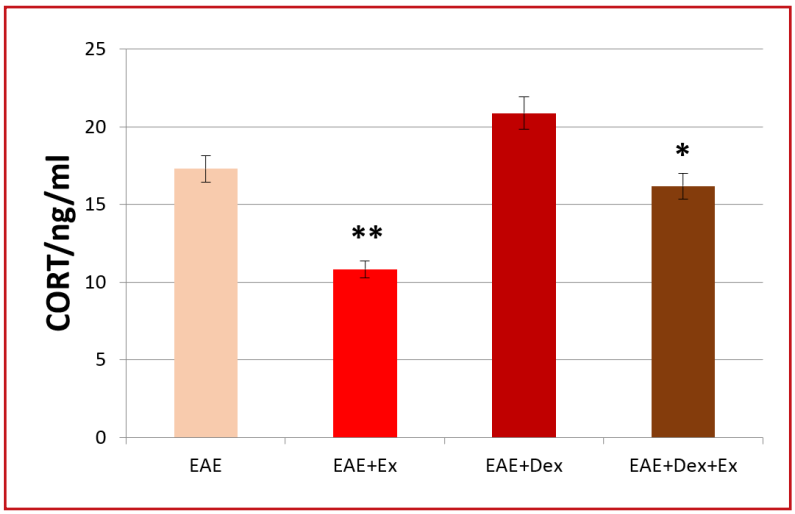

Chart 5. Corticosterone serum concentration for four groups: EAE, EAE + Dex, EAE +Ex, and EAE + Dex + Ex was significantly different between groups p =.05, p > 0.01 respectively. Data are presented as means ± SD

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Discussion

The purpose of the current study was to investigate the effect of regular exercise training with or without dexamethasone treatment on outcomes of spatial memory performance in the animal model of MS, EAE. Specifically, time spent in the selected quadrant, swimming speed, and serum corticosterone were evaluated. The main finding of the study suggests that exercise enhances memory in dexamethasone-treated EAE rats. Further, we confirmed previous studies that demonstrated that dexamethasone significantly delayed the onset of disability. While exercise was able to delay the onset of disability by one day, dexamethasone either with or without adjuvant exercise was able to delay initial signs of disability until 21 days post inoculation. These data support previous work that suggests that exercise can be used as a treatment to delay or ameliorate the disease trajectory in the EAE model of multiple sclerosis (21, 22) and that dexamethasone is an effective therapy in the treatment armament for MS. An adverse effect of the clinical manifestations of MS includes body mass gains. Unfortunately, studies utilizing exercise interventions on EAE models have documented conflicting data regarding body mass. For example, Klaren and colleagues (23) reported that both voluntary wheel running and forced treadmill showed no effect on bodyweight. Further, loss of bodyweight in EAE mice was also reported when subjected to regular swimming exercise (24). The results of this study indicate that bodyweight increased in both the EAE only group and the EAE+Ex groups. These results are in accordance with a previous study published by Patel et al (25) showing that the Ex group of female Lewis rats significantly had a greater body mass compared to sedentary EAE controls. Future work should consider differentiating lean and fat mass to better understand the role of exercise in managing body mass in EAE model.

Decreases in bodyweight is common during therapies with GCs in rodents, particularly when treated with dexamethasone (26). It is suggested that dexamethasone be involved in alterations of leptin level, which is known to regulate the food intake, and is considered as stress hormone (27, 28).Therefore, we can hypothesize that the reduction in body weight observed in this study may be due to leptin although leptin level was not measured in this study. The present study showed that even short-term use of 4mg/kg dexamethasone reduces the bodyweight in the EAE rats. Previous studies have suggested that this reduction in body mass due to dexamethasone can be attributed to altered food intake (29), or changes in bone formation (30).

The effectiveness of GCs treatment in alleviating EAE-induced disability and peripheral inflammation is well demonstrated (9, 31). The results of this study confirm previous reports that EAE rats treated with daily administration of 4mg/kg for 15 days lead to delayed the onset of disability. In our study, dexamethasone treatment was able to delay initial onset of disability till day 21 post inoculation compared to day 12 and 13 for EAE control and EAE-Ex groups, respectively. In addition to the delayed disability, dexamethasone-treated rats also had earlier recovery from signs of disease. Together with the previous work by McCombe and colleagues (20), who demonstrated that 4 mg/kg of Dex administration daily leads to less severe clinical signs and earlier recovery, it is evident that dexamethasone plays a role in modulating the pathophysiology of MS. Similarly, EAE animals treated with high doses of dexamethasone (50 mg/kg) showed significant improvement of the clinical motor signs throughout the disease (32). However, comparisons are hindered by the use of different EAE models and different GCs along with different doses. A potential explanation of the ability of dexamethasone to modify MS pathophysiology is based on dexamethasone's ability to cross the intact blood-brain barrier (33) and is likely to interact with the hippocampal region of the brain.

The hippocampus is the main brain region accountable for cognitive processes and it is susceptible to steroid hormones (34, 35). It plays an important role in memory and operationalizes newly acquired information (36). Elevated

glucocorticoids have been associated with impaired cognition and memory function (37). As such, patients with MS are highly susceptible to memory impairment because of the use of dexamethasone in treating acute exacerbations. The findings of this study indicate that exercise training can improve memory as evident by greater time spent in the selected quadrant and swimming speed at the end of the study. Our results also suggest that exercise can protect against the detrimental effects of dexamethasone treatment in the combination group. The present findings are consistent with Kim and Sung (12), who reported that regular exercise influences the improvement of memory in mice with EAE when subjected to step-down avoidance tasks. Generally, physical activity leads to an increase in hippocampal volume and ameliorates the memory impairments that inflict patients with MS (38, 39) by changing the physiological efficacy of the hippocampus and the prefrontal cortex (40, 41). A plausible explanation could be the effect of exercise on decreasing the circulating levels of corticosterone.

Corticosterone is implicated in impairing memory and cognitive function (42). Results of this study indicate that exercise significantly lowers corticosterone concentration in both EAE rats and EAE rats treated with dexamethasone. The data are consistent with the phenomenon of adaptation and central nervous system plasticity, which indicates that animals subjected to exercise training acclimate and release lower levels of corticosterone, even acutely after a single session of training (43, 44). Indeed, a potential role of corticosterone levels in response to exercise correlates with metabolic changes and neuroendocrine effects (45). A potential explanation for the elevated corticosterone levels in the non-exercising group could be the stress response to the water maze prop test. Most reports suggest that a prop test is equivalent to light exercise (46), and, as such, should not have an effect on increasing corticosterone concentrations the same way moderate intensity exercise does (47). What remains to be understood is what the mechanisms underlying the reduced corticosterone are. Further research is needed to determine if decreased corticosterone concentrations are due to inhibited release of corticosterone, decrease mRNA translation of corticosterone, or decrease transcription at the gene level. Alternative explanations could be the increased metabolism of corticosterone in exercising rodents. Future research should answer these hypotheses.

Another important finding of our study determined that the combination EAE+Dex+Ex group showed a memory improvement as investigated by the hidden probe location during the MWM. Conversely, the EAE+Dex group did not show a significant improvement in memory. To corroborate our results, Nitoon and colleagues (32) also report that EAE-induced animals injected with dexamethasone showed a working memory deficit. This group further rationalized that decreased neuronal effectiveness measured by GCs receptor activity in the hippocampus was also observed in dexamethasone treated EAE animals. On the basis of these findings, it could be suggested that 4mg/kg of dexamethasone treatment leads to increased HPA activity that impairs memory and cognitive function in EAE animals.

Conclusion

To our knowledge, this is the first study using MWM testing in response to regular exercise training during GCs therapy after the recovery stage of EAE disease. The finding of this study strongly suggests that treadmill exercise training regulates the activation of HPA and directly improves memory impairment during EAE, even when dexamethasone is administered. Further, 4 mg/kg of dexamethasone may lead to cause dysfunction of HPA and may lead to increase in circulating corticosterone level. Further, the inclusion of exercise training, either with or in the absence of dexamethasone treatment, improves memory in a rodent model of EAE that is susceptible to such impairment. Therefore, these findings indicate that the treadmill exercise protocol selected in this study was an effective regimen to improve memory and cognition in

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EAE animals during dexamethasone treatment.

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Conflict of interest

The authors declare that they do not have conflict of interest.

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