

Moderate Treadmill Exercise Protects Synaptic Plasticity of the Dentate Gyrus and Related Signaling Cascade in a Rat Model of Alzheimer's Disease

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Abstract The dentate gyrus (DG) of the hippocampus is known to be more resistant to the effects of various external factors than other hippocampal areas. This study investigated the neuroprotective effects of moderate treadmill exercise on early-phase long-term potentiation (E-LTP) and its molecular signaling pathways in the DG of amyloid β rat model of sporadic Alzheimer's disease (AD). Animals were preconditioned to run on treadmill for 4 weeks and concurrently received ICV infusion of $A\beta_{1-42}$ peptides (250 pmol/day) during the third and fourth weeks of exercise training. We utilized in vivo electrophysiological recordings to assess the effect of exercise and/or AD pathology on basal synaptic transmission and E-LTP magnitude of the perforant pathway synapses in urethane-anesthetized rats. Immunoblotting analysis was used to quantify changes in the levels of learning and memory-related key signaling molecules. The AD-impaired basal synaptic transmission and suppression of E-LTP in the DG were prevented by prior moderate treadmill exercise. In addition, exercise normalized the basal levels of memory and E-LTP-related signaling molecules including Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), calcineurin (PP2B), and brain-derived neurotrophic factor (BDNF). Exercise also prevented the reduction of phosphorylated CaMKII and aberrant increase of PP2B seen after E-LTP induction in amyloid-infused rats. Our data suggests that by restoring the balance of kinase-phosphatase, 4 weeks of moderate treadmill exercise prevents DG synaptic deficits and deleterious alterations in signaling pathways associated with AD.

Keywords Treadmill exercise · Alzheimer's disease · Basal synaptic transmission · Early LTP · Memory · BDNF

Introduction

Alzheimer's disease (AD), the leading cause of dementia in the elderly, is an irreversible neurodegenerative disorder in which patients experience memory loss in addition to psychopathic behaviors. Few years ago, the number of people suffering from AD globally was approximately 26.6 million, and this could increase to 106.8 million in 2050 [1]. AD pathology progressively affects various brain regions starting with the hippocampus, a critical area implicated in learning and memory, especially spatial memory [2]. Even though the literature has extensively demonstrated the hippocampus as a whole is where memory starts and temporarily stored, studies have reported different functions of each separate subregion. For example, the dentate gyrus (DG) acts as filter of new information or pattern separation, thus making it an effective gateway into area CA3, a hippocampal region that encodes new spatial information [3–5]. Additionally, alterations along the transverse axis of the hippocampus result in various memory impairments. The septal (dorsal) area of the hippocampus is involved in spatial learning and memory, while its temporal (ventral) counterpart affects motivational and emotional learning [6–8]. Previous studies have shown that in animal models of AD, the DG exhibited reduced hippocampal neurogenesis [9] in addition to a significant increase of both resting and activated microglia, a marker of neuroinflammation [10].

In contrast, regular exercise regimens seem to have protective effects in various brain insults including sleep deprivation, cerebral ischemia, and chronic restraint stress [11–13]. Exercise is beneficial in ameliorating cognitive deficits as shown in learning and memory-specific behavioral tasks including the Morris water maze, radial arm water maze, passive avoidance, and object recognition [11, 14–16]. In addition, synaptic plasticity (i.e., long-term potentiation (LTP)) is rescued by exercise when suppressed by cerebral infarction [17]. Perhaps by increasing the regional rate of cerebral protein

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synthesis, an important element of adaptive plasticity, especially in the DG, exercise modifies both hippocampal and cortical plasticity [18]. One such protein is brain-derived neurotrophic factor (BDNF) whose mRNA and protein levels are often elevated upon various exercise training protocols [19, 20].

Thus, this study aims to investigate the effects of 4 weeks of treadmill exercise on plasticity and signaling pathways of granule cell layers of the DG in a rat model of AD.

Materials and Methods

Animals Adult Wistar male rats (176–200 g) were purchased from Charles River Laboratories, Wilmington, MA, and allowed to acclimatize for 7 days. Rats were housed in Plexiglas cages in groups of six in a climate-controlled room with light on at 7 a.m. and off at 7 p.m. and given free access to regular rat chow and water. All animal manipulations were done following the guidelines from the National Research Council's Guide of The Care and Use of Laboratory Animals and with the approval of the Institutional Animal Care and Use Committee at the University of Houston. Rats were randomly assigned into the following four groups: control, exercise (Ex), amyloid-infused ($A\beta$), and exercise + amyloid-infused (Ex/ $A\beta$).

Treadmill Exercise Training All rats were familiarized with the treadmill environment before the commencement of exercise training. Groups Ex and Ex/ $A\beta$ ran during weekdays on a leveled motorized treadmill for 4 weeks with a customized regimen as described [12, 21–23]. Briefly, the training was divided into sessions in which rats ran two sessions (15 min per session)/day at 10 m/min for 2 weeks, then the speed was increased to 15 m/min during the third and fourth weeks, and rats ran two sessions/day. Confounding factors (e.g., muscle fatigue) were avoided by giving a 5-min break between sessions. Exercised groups were monitored through all sessions and to ensure and encourage continuous running during training a mild foot shock (intensity=0.5 mA), which was not considered to be stressful to the animals [11, 12, 21].

Osmotic Pump Implantation We utilized the ICV infusion of amyloidogenic $A\beta_{1-42}$ peptides to generate the AD model as described [24, 25]. Rats were anesthetized with ketamine (75 mg/kg) and xylazine (2.5 mg/kg) (Webster Veterinary, Devens, MA). Rat groups Ex/ $A\beta$ and $A\beta$ underwent osmotic pump implantation in which $A\beta_{1-42}$ peptides were constantly delivered to the brain via a cannula placed in the right cerebral ventricle (AP −0.3, L 1.2, V 4) at the rate of 0.25 μ l/h for a final concentration of 250 pmol/day for 2 weeks. Control and exercise animals were sham-operated since previous study from our lab showed no difference in behavioral and

electrophysiological testing between vehicle-treated controls and animals that were infused with the inactive amyloid reverse-peptide $A\beta_{42-1}$ [24]. Aseptic techniques were followed during the implantation surgery, and the animals were monitored until full recovery.

Electrophysiology Rats were anesthetized with urethane (1.2 g/kg, Sigma-Aldrich, USA), which is a suitable anesthetic agent in electrophysiology studies [26]. Each rat was positioned in the stereotaxic frame and prepared for recording as described [22, 27]. Two holes were drilled to record population spikes (pspike) from the granule cell layer of the DG area. On the right side of the brain, a concentric bipolar stimulation electrode was placed to stimulate the perforant pathway via the angular bundle (AP −8, L 4.7, V 1.2), and a glass capillary recording electrode was positioned to record from the granule cell layer (AP −3, L 2, V 3.5). The electrode position was adjusted if necessary to obtain a maximal pspike response. Input/output (I/O) curves were constructed to assess changes in basal synaptic transmission of the DG area by plotting different stimulus intensities (input) versus the field excitatory postsynaptic potential (fEPSP) slope (output). Then, a baseline was established by giving a test stimulus (30 % of maximum) every 30 s for 20 min. Early-phase long-term potentiation (E-LTP) was evoked by a single train of high frequency stimulation using our published protocol [11, 22]. Changes in fEPSP slope and pspike amplitude correspond to changes in synaptic strength and the number of neurons that reached threshold and fired respectively.

Western Blotting Quantifications of changes in the levels of key signaling molecules pertaining to learning, memory, and E-LTP were done under basal (no E-LTP induction) and stimulated (after E-LTP induction) conditions.

Rats in all groups were euthanized by a lethal injection of urethane into the heart. Immediately after the end of E-LTP recording, the right DG regions were isolated and further divided into the septal and temporal regions. We consider the temporal part of the DG area as unstimulated internal control since most of the stimuli go to the septal portion of the same right hippocampus [28]. Therefore, to reduce variations, after normalizing levels of molecules in each side against glyceraldehyde phosphodehydrogenase (GAPDH), levels of signaling molecules were expressed as ratio of the levels in the septal side to those of the temporal side of DG. Unstimulated samples (no E-LTP induction) were isolated from control rats and handled in a similar way.

The tissues were lysed in a premixed buffer and prepared for gel electrophoresis as described [22]. We used a microBCA assay kit (Pierce Chemical Rockford, IL) to estimate the amount of total protein in each sample. Approximately 10–15 μ g of total protein in each sample was loaded onto the 48 E-PAGE gel (Invitrogen Corp.,

Carlsbad, CA) and electrophoresed for 30 min. Then, the proteins were blotted on a PVDF membrane using the iBlot transfer system (Invitrogen Corp., Carlsbad, CA). To measure the levels of a protein, we incubated with polyclonal or monoclonal primary antibodies as outlined below, and the immunoreactive bands were detected by a horseradish peroxidase-conjugated secondary antibody. Subsequently, the blots were developed using chemiluminescence reagent, detected in an Alpha Innotech imaging system, and quantified by densitometry using AlphaEase software and expressed as a ratio to that of the GAPDH blot.

The protein of interest was probed using specific primary antibodies with conjugation of horseradish peroxidase secondary antibodies. Antibody dilutions were done according to the following: mouse monoclonal anti-p-CaMKII (1:500), rabbit polyclonal anti-t-CaMKII (1:1,000), rabbit polyclonal anti-BDNF (1:500), rabbit polyclonal anti-PP2B (1:1,000), rabbit polyclonal anti-GAPDH (1:1,000), and secondary anti-mouse/rabbit antibodies (1:5,000). These antibodies were purchased from Santa Cruz Technology, Dallas, TX, and Cell Signaling Inc., Boston, MA.

Results

The Neuroprotective Effect of Treadmill Exercise on Basal Synaptic Transmission in the DG Area

We evaluated the effect of AD pathology and/or exercise on basal synaptic transmission in the DG area by evaluating the I/O curves. The I/O curve will change when synaptic strength is altered [29, 30]. Our data indicated that A β rats exhibited impaired basal synaptic transmissions in the DG area of the hippocampus. The A β rats exhibited a significantly lower fEPSP slope at all stimulus intensities than those of the control, exercise, and Ex/A β rats ($p=0.001$) (Fig. 1a). For example, at minimal intensity, the fEPSP slope of A β rats was 2.585 ± 0.286 mV/ms, which was much smaller than that of Ex/A β rats (4.215 ± 0.359 mV/ms), which was similar to that of control (4.62 ± 0.323 mV/ms) and exercise rats (3.979 ± 0.04 mV/ms). Thus, it seems that AD pathology severely impairs basal transmissions of the perforant synapses, while regular moderate treadmill exercise protects these synapses.

In addition to I/O curves, basal synaptic transmissions in the DG area were further assessed by the magnitude of the voltage required for producing the minimal, 30 % of maximum, and maximal responses. As demonstrated in Fig. 1b, A β rats required a mean voltage of 6.26 ± 0.189 mV to elicit a minimal response, which was markedly higher compared to Ex/A β rats (5.14 ± 0.299 mV) ($p=0.05$), and this was not different from those of control (4.475 ± 0.382 mV) and exercise groups (3.58 ± 0.18 mV). Together, these data suggested

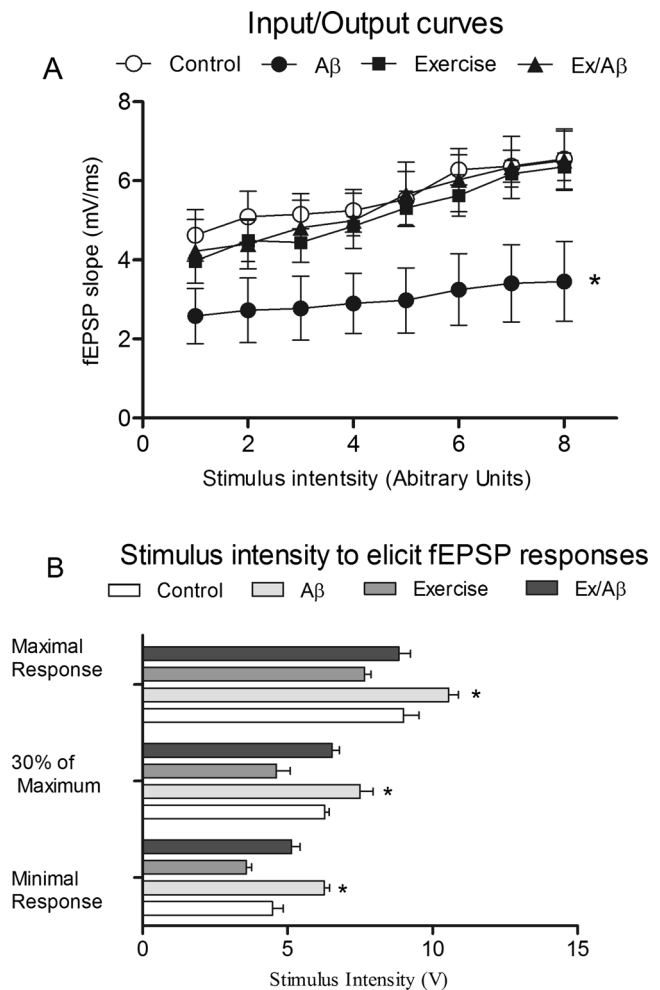


Fig. 1 AD pathology impairs basal synaptic transmission in the DG area of A β rats, while moderate treadmill exercise prevents this impairment. **a** The input–output (I/O) curves are indices of synaptic responses (i.e., field excitatory postsynaptic potential (fEPSP) slope) constructed from gradual increases in stimulus intensity. **b** Stimulus intensity required to elicit minimal, 30 % of maximum, and maximal response. The I/O curve of A β rats is altered, while that of Ex/A β rats is not different from controls. A β rats also require a significantly stronger voltage to elicit the same response compared to control, exercise, and Ex/A β rats. Asterisk indicates significant difference compared to other groups at all stimulus intensities. Values are mean \pm SEM, $n=4$ –6 rats/group

that the impaired basal synaptic transmission caused by AD pathology was prevented by prior regular treadmill exercise.

Regular Treadmill Exercise Prevented AD-Induced Suppression of E-LTP in Perforant Path Synapses

We evaluated the effects of 4 weeks of treadmill exercise on electrical stimulation-evoked E-LTP magnitude in the DG area of the hippocampus. Repetitive high-frequency stimulation (HFS) induced a robust postsynaptic response of the perforant synapses in all groups except the A β group. One hour after E-LTP induction, the fEPSP slope in A β rats ($99.618\%\pm7.417$ of baseline) was significantly different than

all other groups ($p=0.001$). However, with exercise, the fEPSP slope in Ex/A β rats was 138.434 ± 9.136 , which was similar to those of control (142.044 ± 4.835) and exercise (135.606 ± 6.569) rats (Fig. 2a). It is notable that although exercise protected rats in the Ex/A β group, it did not potentiate the response of normal rats (Fig. 2a). Interestingly, unlike in area CA1 [22], neither amyloid infusion alone nor combined with exercise training altered the pspike amplitude indicating that the number of neurons that reached threshold and fire in the DG area was similar across

all groups (control 225.786 ± 14.311 , A β 254.697 ± 34.631 , exercise 231.245 ± 29.616 , Ex/A β 232.962 ± 40.488) (Fig. 2b).

Reduction of Phosphorylated CaMKII in A β Rats Was Prevented by Regular Treadmill Exercise

In the DG area, the basal level of p-CaMKII in A β rats was 0.291 ± 0.068 , which was markedly lower than that in control (1.0 ± 0.063), exercise (0.75 ± 0.083), and Ex/A β (1.021 ± 0.116) rats ($p=0.05-0.01$) (Fig. 3a). However, the levels of basal total CaMKII in the DG area were similar across all groups (control 3.546 ± 0.414 , A β 3.14 ± 0.496 , exercise 2.92 ± 0.294 , Ex/A β 3.243 ± 0.448) (Fig. 3b). Hence, the p-CaMKII/t-CaMKII ratio of A β rats (0.083 ± 0.015) was significantly smaller compared to those of control rats (0.302 ± 0.047), Ex/A β rats (0.339 ± 0.041), and exercise rats (0.281 ± 0.032) ($p=0.01-0.05$) (Fig. 3c) suggesting impairment of phosphorylation.

A single train of HFS robustly increased the level of p-CaMKII in stimulated (S-) control (1.629 ± 0.115), S-exercise (1.911 ± 0.238), and S-Ex/A β rats (2.023 ± 0.268) compared to unstimulated control (1.03 ± 0.151) in the DG area ($p=0.01-0.05$) (Fig. 4a). In contrast, the same E-LTP induction protocol failed to increase the p-CaMKII level in A β rats (0.603 ± 0.175). After HFS, the levels of t-CaMKII in all stimulated groups were similarly elevated (S-control 1.779 ± 0.5043 , S-A β 1.731 ± 0.292 , S-exercise 1.965 ± 0.4194 , and S-Ex/A β 1.869 ± 0.6925) compared to those in the unstimulated control (0.8717 ± 0.2421) ($p=0.05-0.01$) (Fig. 4b). As a result, the p-CaMKII/t-CaMKII ratio in the DG area of S-A β rats (0.3933 ± 0.2784) was significantly lower than those of all other groups (S-control 1.223 ± 0.1201 , S-exercise 1.432 ± 0.3682 , and S-Ex/A β 1.256 ± 0.2239) including unstimulated control (1.041 ± 0.3477) ($p=0.01-0.05$) (Fig. 4c).

The Aberrant Increase of Calcineurin Rats Was Prevented by Regular Treadmill Exercise

Calcineurin (PP2B) is a phosphatase that inactivates CaMKII, thus returning the constitutive activity of CaMKII back to normal. Western blot analysis revealed that in the DG area, there was a significant increase in the basal level of calcineurin (1.314 ± 0.399) in A β rats compared to other groups ($p=0.01-0.05$). However, the basal levels of calcineurin in the DG area of Ex/A β rats (0.6516 ± 0.142) were similar to those of control (0.6704 ± 0.146) and exercise (0.7473 ± 0.1555) rats (Fig. 5a). Overall, it seems that by preventing the phosphatase-kinase imbalance, regular exercise prevents synaptic deficits caused by AD pathology.

The levels of calcineurin (PP2B) were increased after E-LTP induction in the perforant synapses in S-control (1.601 ± 0.3312) and S-A β (1.539 ± 0.3317) rats compared to

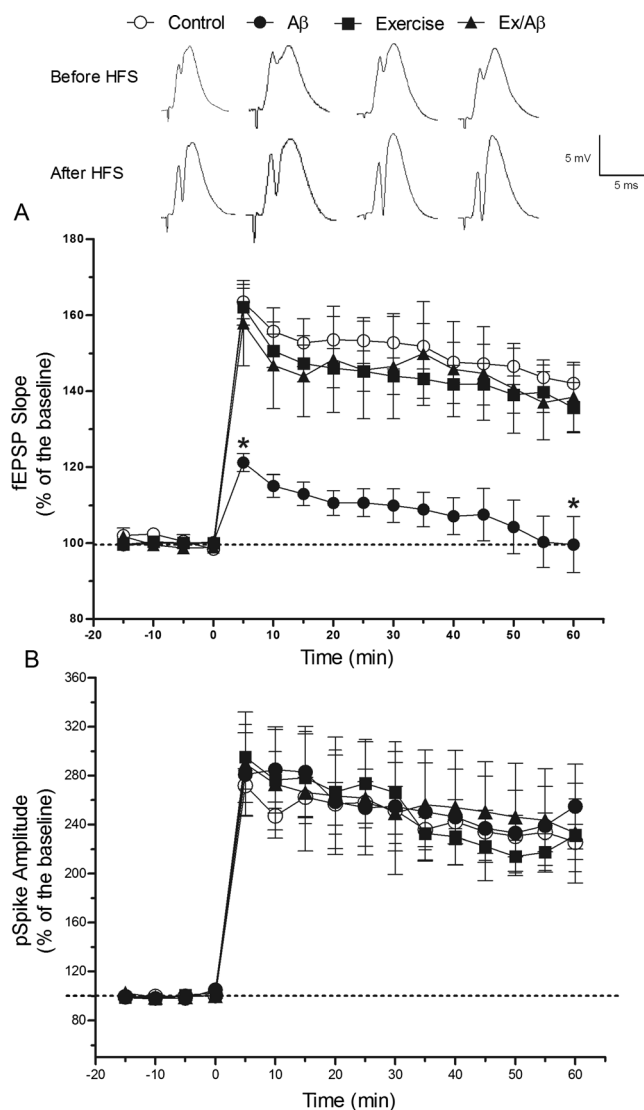


Fig. 2 Early-phase LTP (E-LTP) is measured in the DG area of the hippocampus: fEPSP slope (a) and pspike amplitude (b). Single train of HFS (applied at time zero) evokes E-LTP of perforant synapses in anesthetized rats. The fEPSP slope of A β rats is significantly lower compared to other groups, while Ex/A β rats exhibit a similar fEPSP slope compared to that of control and exercised rats. Surprisingly, the pspike amplitudes of all rats including rats with amyloid peptides infusion alone are the same. Each point is the mean \pm SEM of five to six rats. Points between the two asterisks indicate significant difference from all groups

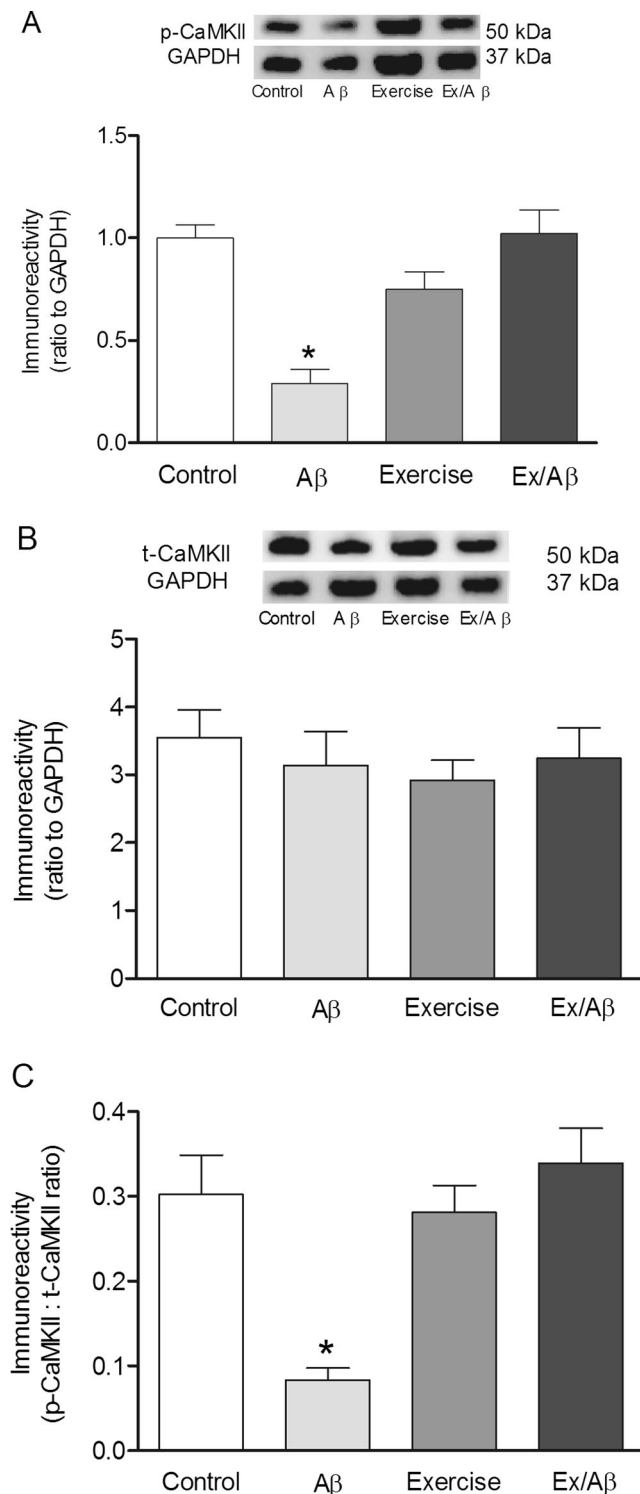


Fig. 3 Basal levels of p-CaMKII (a), t-CaMKII (b), and p-CaMKII/t-CaMKII ratio (c) in the DG area. The basal levels of p-CaMKII in Aβ rats are significantly decreased compared to other groups, while the levels of t-CaMKII are similar across all groups. Thus, the p-CaMKII/t-CaMKII ratio of Aβ rats is significantly smaller than those of control, exercise, and Ex/Aβ rats, which indicates that AD pathology targets primarily the phosphorylation process not the protein synthesis or total protein pool of CaMKII. Regular treadmill exercise prevents the reduction in p-CaMKII basal levels and p-CaMKII/t-CaMKII ratio of Aβ rats. Asterisk indicates significant difference from control, exercise, and Ex/Aβ ($p < 0.05$). Values are mean \pm SEM, $n = 4$ –6 rats/group. Insets are representative blots

Regular Treadmill Exercise Increases the Protein Expression of BDNF in Exercised Rats in the DG Area

We examined the effect of AD pathology and/or moderate treadmill exercise on the levels of BDNF in the DG area under basal condition and after E-LTP expression. The DG area is one of the rare brain regions in which neurogenesis occurs. This critical area of the brain also houses numerous neurotrophic factors including BDNF. Our data revealed that in the DG area, regular exercise significantly increased the basal levels of BDNF in the two exercised groups (exercise 1.758 ± 0.5121 , Ex/Aβ 1.806 ± 0.6664). However, the basal level of BDNF in Aβ rats was similar to that of sedentary controls (control 1.0 ± 0.1601 , Aβ 0.9145 ± 0.1682) (Fig. 6a).

We also measured BDNF dimer levels in the DG area after E-LTP induction. Our data showed that HFS significantly upregulated the BDNF levels in S-exercise (2.315 ± 0.6279) and S-Ex/Aβ (2.067 ± 0.5055) groups compared to unstimulated control (1.0 ± 0.1959), S-control (1.196 ± 0.4989), and S-Aβ (1.155 ± 0.5027) groups ($p = 0.05$) (Fig. 6b).

Discussion

Hippocampal synapses, including the generally insult-resistant granule cells of the DG, are quite vulnerable in AD pathogenesis. For example, Aβ_{1–42} potentially inhibits LTP in the DG area in vitro and in vivo, probably by interfering with *N*-methyl-D-aspartate (NMDA) receptor signaling [31, 32]. Other studies also demonstrate severe impairment of both early- and late-phase LTP in hippocampal slices of transgenic AD mice compared to wild-type controls [33–35]. Consistent with these findings, our data revealed that in the DG area, 2 weeks of infusion of Aβ_{1–42} shifted the input/output (I/O) curves of Aβ rats to the right, indicating impaired basal synaptic transmissions. Studies proposed that depression of basal synaptic transmission and LTP in AD is probably due to the activation of presynaptic Ca²⁺-activated K⁺ channels, an important regulator of cell excitability and transmitter release [36]. Under the effect of amyloid infusion, perforant pathway

unstimulated control (1.017 ± 0.1626 ; $p = 0.05$) (Fig. 5b). However, the levels of PP2B during E-LTP expression in the DG area of the exercised groups (exercise and Ex/Aβ) were similar to those of unstimulated control (S-exercise 1.041 ± 0.4078 , S-Ex/Aβ 1.083 ± 0.2347) (Fig. 5b).

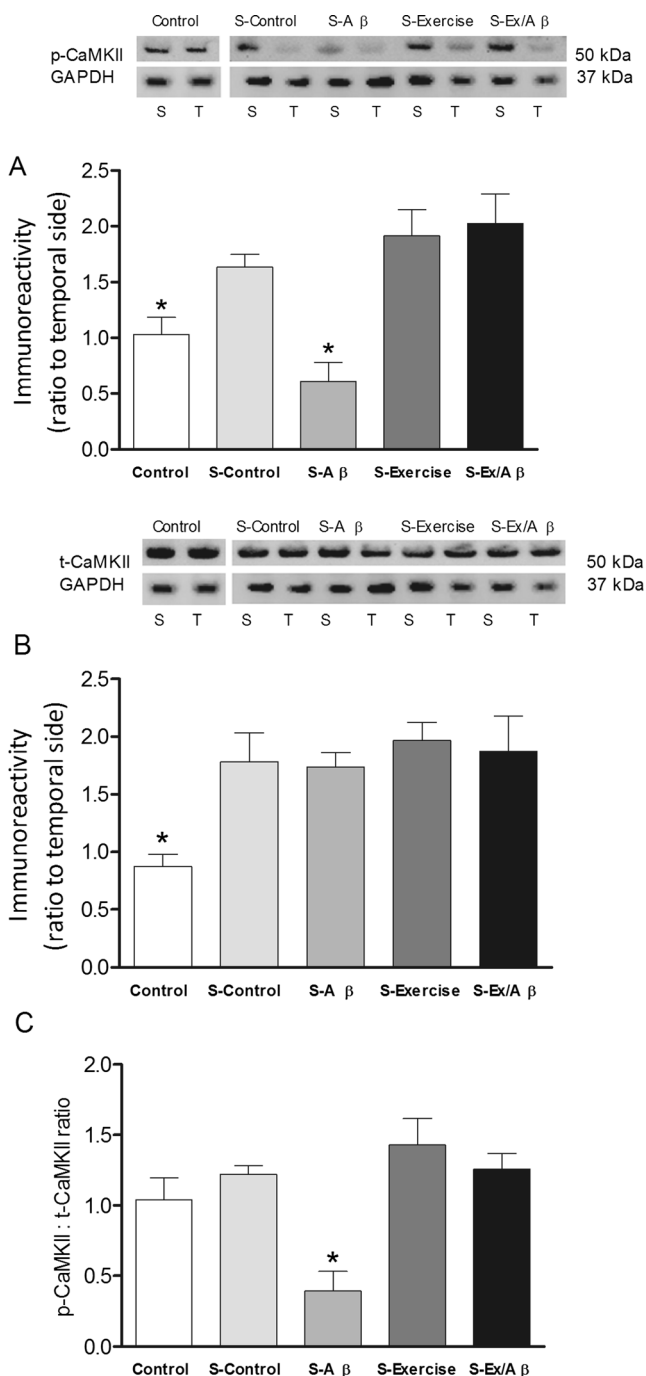


Fig. 4 Levels of phosphorylated (p)-CaMKII (a), total (t)-CaMKII (b), and the p-CaMKII/t-CaMKII ratio (c) in the DG area after E-LTP induction (stimulated (S-) condition). Compared to the unstimulated control group, HFS increases the level of p-CaMKII in all groups except the A β rats and significantly increased the levels of t-CaMKII in all groups. Thus, after HFS, the p-CaMKII/t-CaMKII ratio was significantly lowered in A β rats compared to all other groups including unstimulated control rats. Regular exercise prevented AD-induced reduction in p-CaMKII level. Asterisk in **a** indicates significant difference from stimulated (S-) control, S-exercise, and S-Ex/A β , and in **b**, **c** indicates significant difference from all other groups ($p < 0.05$). Values are mean \pm SEM, $n = 4-6$ rats/group. Insets are representative blots. S septal portion, T temporal portion of the hippocampal DG area

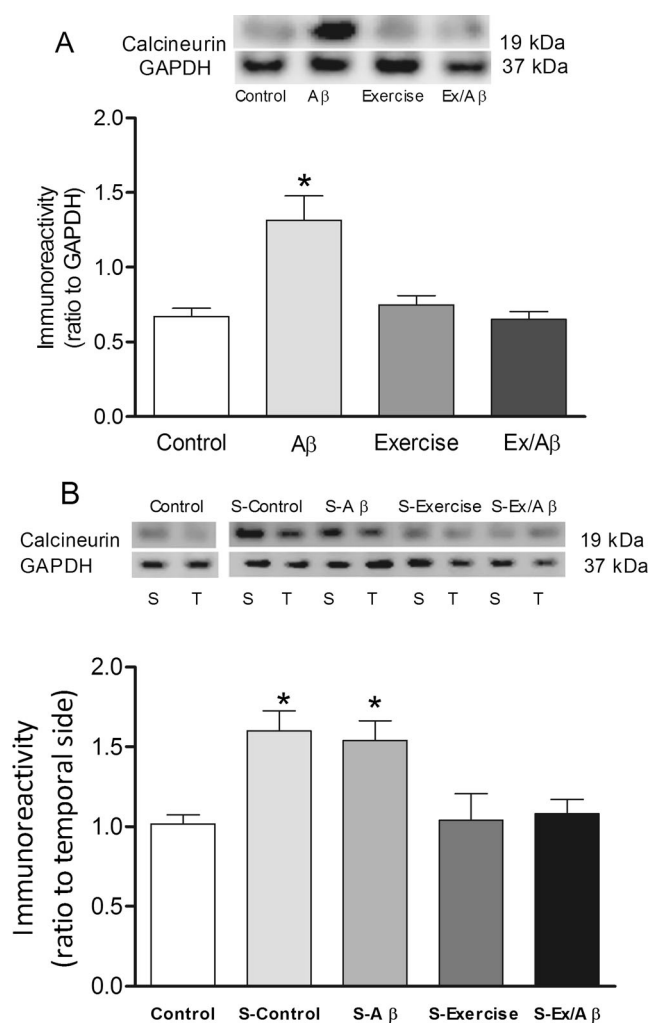


Fig. 5 Levels of calcineurin (PP2B) under the basal (a) and after E-LTP induction stimulated (S-) condition (b) in the DG area. Exogenous administration of A β_{1-42} peptides increases the basal levels of PP2B in A β rats. Additionally, HFS application increases the expression of calcineurin in S-control and S-A β rats, which are significantly higher than those of S-exercise, S-Ex/A β , and unstimulated control rats. Moderate treadmill exercise significantly reduces the basal and E-LTP induced increase levels of PP2B in Ex/A β rats. Asterisk in **a** indicates significant difference from all groups and in **b** indicates significant difference from unstimulated control, S-exercise, and S-Ex/A β rats, $p < 0.05$. Values are mean \pm SEM, $n = 4-6$ rats/group. Insets are representative blots. S septal portion, T temporal portion of the hippocampal DG area

synapses require much higher voltage in order to elicit the same response in control animals. We also observed suppression of E-LTP in these synapses, which highly correlates with our behavioral findings reported in previous publications [22, 23].

Our data indicated that 4 weeks of moderate treadmill exercise protected the perforant synapses against the deleterious effect of AD pathology. The right side shift of the I/O curve observed in the DG area of A β rats was completely absent in animals with exercise training as the I/O curve of Ex/A β rats was similar to that of control rats and the voltages

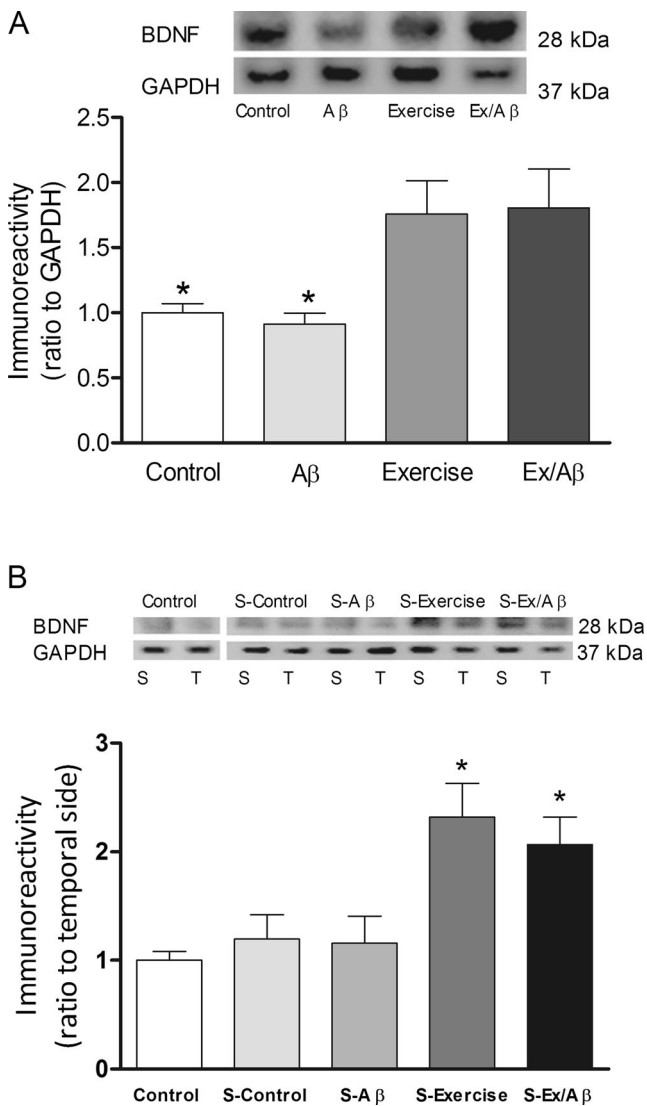


Fig. 6 Levels of BDNF in the DG area under the basal (a) and after E-LTP induction stimulated (S-) condition (b). Four weeks of treadmill exercise significantly increases the basal levels of BDNF in exercised rats including Ex/A β rats. HFS does not increase the level of BDNF in S-control and S-A β rats but significantly elevates that in S-exercise and S-Ex/A β rats. Asterisk in **a** indicates significant difference from exercise and Ex/A β rats and in **b** indicates significant difference from unstimulated control, S-control, and S-A β , $p < 0.05$. Values are mean \pm SEM, $n = 4-6$ rats/group. Insets are representative Western blots. S septal portion, T temporal portion of the hippocampal DG area

required to elicit minimal and maximal response in Ex/A β and control rats were not different indicating protection of basal synaptic transmission by exercise. Additionally, we found that the DG area of A β rats exhibited markedly lower fEPSP slope throughout the E-LTP recording period, while prior exercise abolished this negative effect. This clearly indicates that exercise prevented impairment of basal synaptic transmission as well as synaptic plasticity in A β rats. In addition to fEPSP slope measurement, after E-LTP induction, we also evaluated the pspike amplitude, which represents the number of neurons

that reach threshold and fire. To our surprise, the pspike amplitude in the DG area of A β rats remained unaltered compared to all other groups after HFS. This is in contrast to our previously reported findings in area CA1 in which HFS failed to induce E-LTP in A β rats, and even the pspike amplitude in Ex/A β rats was not fully restored to control level [22].

The lack of effect of A β infusion on pspike amplitude in DG may be attributed to the known unusual properties and resilience of the diminutive granule cells of the DG area. It is recognized that granule cells of the DG area are more resistant to stress, ischemia, anoxia, and seizure susceptibility than are the large pyramidal cells of area CA1 [37–39]. Additionally, there are major differences in the density and/or distribution of NMDA receptors and T-type calcium channel in the CA1 and DG areas [40]. Remarkably, the granule cell and the pyramidal neuron differ in their capacity to buffer intracellular calcium. Calbindin-D28K is a member of a calcium-binding protein family that is present in many brain areas, including area CA1 and DG of the hippocampus [41, 42], and is very important for neuronal function and plasticity [43]. Calbindin appears at high concentration in virtually all granule cells of the DG, but it is present in less than one third of the neurons in area CA1 [44, 45, see 46 for review]. Interestingly, prolonged stimulation of rat DG area in vivo leads to degeneration only of those neurons that lack immunoreactivity for calbindin [47]. Thus, activity-induced excessive increase in intracellular calcium can lead to cell deterioration, but neurons that have the capacity to capture free calcium would be less vulnerable to damage [47].

Currently, the mechanisms of the neuroprotective effect of exercise on AD synapses remain elusive. One potential factor involved in this process is CaMKII whose vital function is the constitutive kinase activity during synaptic plasticity and memory processes. CaMKII is abundantly distributed in hippocampal postsynaptic density and memory center of *Drosophila* [48, 49], which can act as a “molecular memory switch” [50, 51]. Pharmacological and genetic manipulations of CaMKII expression and activity result in deficits in LTP and memory [52, 53]. Once activated, CaMKII can only be turned off by phosphatases suggesting the important role of phosphatases in CaMKII-related signaling cascades, especially in learning and memory. For example, in a transgenic mouse model of AD, inhibition of protein serine–threonine phosphatases fully restores AD-induced impairment of LTP [54, 55]. Our findings suggest that 4 weeks of treadmill exercise prevented the elevated levels of calcineurin in our AD model. Perhaps by keeping the kinase–phosphatase balance closed to physiological condition, exercise is able to maintain synaptic plasticity in the DG area even in serious conditions such as AD pathology.

The neuroprotective effect of exercise is at once intriguing and enigmatic. There is evidence for communication between

skeletal muscles and the brain, for example, it has been reported that there is an increase in the discharge frequency of hippocampal CA1 neurons when the subject's running velocity increases [56]. Studies proposed that active skeletal muscles could secrete numerous factors that trigger a protective effect on the brain [57, 58]. Although the exact molecular mechanism responsible for the cross talk between the skeletal muscles and CNS remains uncertain, evidence of involvement of particular molecular agents has emerged. Exercise upregulates expression of the mitochondrial molecule, uncoupling protein 2 (UCP2), which protects neuronal mitochondria from oxidative stress, enhances ATP production, and regulates normal calcium level [59]. In addition, UCP2 can also modulate BDNF signaling and its downstream mediators such as CREB and CaMKII [60]. Regular exercise also increases the expression of several cognition-related molecules including BDNF and CaMKII, whose levels are severely curtailed by AD pathology. The reduction in the level of p-CaMKII is due to AD-induced impairment of CaMKII phosphorylation [24, 30, 61], at least partly by increasing calcineurin levels. Thus, the disease seems to target the phosphorylation mechanism enabled by p-CaMKII, which is essential for the expression of LTP and cognitive function.

The molecule that is believed to play an important role in the beneficial effect of exercise is BDNF [62]. A nerve growth factor, BDNF is a critical molecule responsible for neuronal development and synaptic plasticity and is dramatically up-regulated by exercise [12, 63]. Interestingly, our work shows that neither the basal nor stimulated levels of BDNF were reduced in area CA1 [23] or the DG area in A β rats indicating a possible compensatory mechanism to maintain this important molecule at least during the initial effect of toxic amyloid peptides. In the DG area, as in the previously reported area CA1 [23], the exercised groups, including exercise alone and Ex/A β rats, showed a marked elevation in the basal and stimulated levels of BDNF compared to those of the sedentary control. It has been suggested that BDNF is probably produced in peripheral tissues [64] where it crosses the blood–brain barrier in small amounts [65] and augments available BDNF in the central nervous system to produce a beneficial effect on brain function.

Clearly, there is ample evidence for the positive effect of exercise in a variety of brain disorders; however, an important question remains unclear: How does muscle activity translate into a beneficial effect on the brain? Although a significant amount of BDNF is produced in skeletal muscles at rest and particularly during prolonged exercise in healthy male volunteers, it is not released into the circulation but stays in the muscle, perhaps to serve in an autocrine and/or paracrine capacity [66]. Thus, BDNF is not the messenger mediating the cross talk between the active skeletal muscle and the brain. Possible messengers between active skeletal muscles and the brain have been discussed in a number of topical reviews [see 67, 68 for review].

The finding that impairment of basal synaptic transmission and suppression of E-LTP seen in the AD rat model is prevented by regular exercise correlates with the absence of A β -induced deleterious changes in levels of synaptic plasticity-related molecules (CaMKII, PP2B, BDNF) in the exercised rats. Furthermore, failure of repetitive stimulation to increase the levels of p-CaMKII and BDNF in the DG area of A β rats is totally prevented by our exercise regimen. Together, these results suggest that there is an upstream mechanism that mediates the beneficial effects of exercise on the perforant path synapses.

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Conflict of Interest The authors disclose no conflict of biomedical or financial interest.

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