Molecular Neurobiology (2019) 56:4980–4987
https://doi.org/10.1007/s12035-018-1425-x

Effect of Exercise and Aβ Protein Infusion on Long-Term Memory-Related Signaling Molecules in Hippocampal Areas

Karim A. Alkadhi[1] · An T. Dao[1]

Received: 18 September 2018 / Accepted: 5 November 2018 / Published online: 14 November 2018

Abstract

Alzheimer's disease (AD) results from over-production and aggregation of β-amyloid (Aβ) oligopeptides in the brain. The benefits of regular physical exercise are now recognized in a variety of disorders including AD. In order to understand the effect of exercise at the molecular level, we studied the impact of exercise on long-term memory-related signaling molecules in an AD rat model. The rat model of AD (AD rat) was produced by 14-day osmotic pump infusion of i.c.v. 250 pmol/day $A\beta_{1-42}$. The effects of 4 weeks of regular rodent treadmill exercise on the protein levels of CREB, CaMKVI, and MAPK-ERK1/2 in this model were determined by immunoblot analysis in the CA1 and dentate gyrus (DG) areas of the hippocampus, which is among the first brain structures impacted by AD. Aβ infusion caused marked reductions in the basal protein levels of CaMKVI and phosphorylated CREB without significantly affecting total CREB levels in both CA1 and DG areas. As predicted, our exercise regimen totally prevented these effects in the brains of exercised AD rats. Surprisingly, however, neither Aβ infusion nor exercise had any significant effect on the levels of phosphorylated or total ERK in the CA1 and DG areas. Additionally, exercise did not increase any of these molecules in healthy normal rats, which indicated a protective effect of exercise. These findings suggest that CaMKIV is likely a major kinase for phosphorylation of CREB. Therefore, regular exercise is highly effective in preventing the effects of AD even at the molecular level in both areas of the hippocampus. Considering the well-known resistance of the DG area to insults relative to area CA1, the present findings revealed similar molecular vulnerability of the two areas to AD pathology.

Keywords Rat AD model · Amyloid-beta · Regular exercise · Signaling molecules · ERK1/2 · CaMKIV

Introduction

The mitogen-activated protein kinase (MAPK-ERK) and Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) are known to rigorously participate in long-term memory consolidation and late-phase long-term potentiation (L-LTP) by their ability to phosphorylate and activate cAMP response element binding protein (CREB) [1]. Mice carrying CaMKIV mutation display impaired long-term memory and L-LTP as well as attenuated CREB phosphorylation without affecting early-phase LTP (E-LTP), learning acquisition, or short-term memory [2].

Similar in structure to that of calcium calmodulin kinase II (CaMKII), CaMKIV has a Ca^{2+}/calmodulin (CaM) site; once occupied, the complex will trigger kinase activity. Moreover, phosphorylation of the CaM-bound CaMKIV at a threonine residue on its activation loop sustains CaMKIV activity on an autonomous mode, which is necessary for transcriptions [3]. Experimentally, multiple high-frequency stimulation (MHFS) activates the calcium signaling pathway through which CaMKIV phosphorylates CREB. Transgenic mice lacking CaMKIV show impaired L-LTP, long-term memory, and CREB phosphorylation with normal E-LTP and short-term memory [4–6]. In contrast, increased expression of CaMKIV can enhance LTP and spatial learning and memory and can even salvage impaired memory [7].

The CREB molecule is a transcription factor important for expression of L-LTP and memory formation [8–10]. CREB is activated through phosphorylation at serine 133 by several kinases [11]. Elevated intracellular Ca^{2+} through learning activity or depolarization by tetanic stimulation activates CaMKIV, which eventually phosphorylates CREB triggering transcriptional activity. Mice expressing genes with increased level and activity of CREB show an enhanced memory and

✉ Karim A. Alkadhi
kalkadhi@uh.edu

[1] Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX 77204-5037, USA

lower threshold for expression of LTP [12, 13]. In contrast, inhibiting CREB or blocking its phosphorylation by drugs or genetic manipulations reduces LTP magnitude and increases long-term depression (LTD) expression [14, 15], and even causes neuronal death [16].

Long-term memory consolidation involves activation of several kinases including ERK1/2 as well as de novo gene expression and protein synthesis [17, 18]. ERK1/2 activation plays a pivotal role in the associative and contextual memory consolidation [19, 20]. ERK1/2 general structure consists of a catalytic loop domain and a hydrophobic skeleton. Phosphorylation of two residues in the activation segment changes ERK1/2 from an inactive to active state [21]. ERK1/2 acts on several targets including transcription factors, which results in promoting eventual transcription process [22, 23].

Several signaling molecules including brain-derived neurotrophic factor (BDNF), G protein coupled receptors, tyrosine kinases, and repetitive stimulation can activate the ERK pathway. This pathway is involved in hippocampal LTP induction; for example, blocking ERK1/2 activation leads to a marked attenuation of LTP induction but does not affect the expression of established LTP [24]. Additionally, LTP-induced phosphorylation of ERK1/2 can directly modulate CREB signaling and its related transcription processes [1].

Methods

Adult male Wistar rats (176–200 g bw) were kept in Plexi glass cages in a climate-controlled room (25 °C) on a 12 h/12 h, light/dark cycle, and provided with rat chow diet and water ad libitum. All animal experiments were performed according to the instructions from National Research Council's Guide of The Care and Use of Laboratory Animals and with the approval of the University of Houston Institutional Animal Care and Use Committee (IACUC). After a 7-day acclimatization period, rats were randomly assigned into four groups: control, exercise, amyloid-infused (Aβ), and amyloid-infused/exercised (Ex/Aβ).

Exercise Training

After familiarizing the exercise and Ex/Aβ rat groups with the treadmill environment, these rats were trained to run on a motorized rodent treadmill between 9:00 a.m. and 4:00 p.m., 5 days/week for 4 weeks as described [25]. Rats were made to ran 15-min sessions with a 5-min rest break between sessions. In the first 2 weeks of exercise, rats ran two sessions at a speed of 10 m/min, then the speed and number of sessions were increased in the 3rd and 4th weeks, where rats ran three and four sessions respectively at a speed of 15 m/min. Control and Aβ rats were kept on idle treadmills for the same period of time as the exercise and Ex/Aβ rat.

Osmotic Pump Implantation

The Aβ and Ex/Aβ groups were implanted with 14-day osmotic mini-pumps (Alzet, Cupertino, CA) pre-loaded with $A\beta_{1–42}$ peptide (AnaSpec Inc., San Jose, CA). To prevent aggregation in the pump, the peptide was dissolved in a solution containing 64.9% distilled water, 35% acetonitrile, and 0.1% trifluoroacetate (TFA). The pumps were assembled according to the manufacturer's instructions, filled with $A\beta_{1–42}$ solution (250 pmol/day), and primed in isotonic normal saline at 37 °C overnight. Rats were anesthetized with an i.p. mixture of ketamine (75 mg/kg) and xylazine (2.5 mg/kg). Each rat was positioned in a stereotaxic frame with nose bar adjusted at 0.0 and the implantation site was shaved and cleansed with alcohol. The skull was exposed by a 2.5-cm midline incision of the skin starting behind the eyes. The infusion cannula was inserted into the right lateral cerebral ventricle (AP − 0.3, L 1.2, V 4) through a predrilled burr hole and fastened with dental cement. The body of the pump was placed in a subdermal pocket in the back of the rat, and the incision was closed with wound clips and disinfected with tincture of iodine, 60× diluted chlorohexidine, and triple antibiotic ointment. Rats in the control and exercise groups underwent a similar operation using similar concentration of the inactive reverse peptide $A\beta_{42–1}$.

Western Blotting: Hippocampus Dissection, Tissue Homogenization, And Protein Estimation

After euthanizing anesthetized rat with a lethal injection of urethane into the heart, the brain was removed and CA1 and DG areas of the right hippocampus were dissected out and processed as reported [26, 27]. The CA1 and DG tissues were homogenized separately with 200 μL lysis buffer cocktail containing; 50 mM Tris–HCl (pH = 7.4), 1% NP-40, 0.1%SDS, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 5 mM $Na_4P_2O_7$, 100 μg/ml PMSF, 40 mM β-glycerophosphate, 1 mM PMSF, and protease. The samples were then sonicated three times, 5 s/time (Vibra cell, Sonics and Materials Inc., Newtown, CT). The amount of protein in each sample was estimated using microBCA assay kit by constructing concentration curves from standard samples (Pierce Chemical Rockford, IL). Approximately 10–15 μg of total protein/sample was processed via a high throughput system E-PAGE 48 (Invitrogen Corp. Carlsbad, CA). The proteins were transferred onto a PVDF membrane using a dry blot system (Invitrogen Corp. Carlsbad, CA). Specific primary antibodies were used to detect proteins followed by conjugation with horse-radish peroxidase secondary antibodies. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. Antibody dilutions were detailed as reported [25]. All antibodies were purchased from Santa Cruz Technology and from Cell Signaling Inc., Boston, MA.

The protein bands were visualized via chemiluminescence reagents (Santa Cruz Biotechnology) and AlphaEase software and quantified by densitometry.

Statistical Analysis

To compare two groups, we used unpaired *t* test and to compare all groups, we used one-way analysis of variance (ANOVA) followed by Tukey post hoc test. All statistical analyses were performed with GraphPad Prism7 software. Statistical significance is indicated when $P < 0.05$. Data were expressed as mean ± S.E.M.

Results

Phosphorylated and Total CREB in CA1 and DG Areas

Since CREB is an essential regulator of long-term memory and the underlying synaptic plasticity [28, 29], it is expected that the level of this signaling molecule would be highly impacted by Aβ. We previously reported AD pathology-related marked suppression of L-LTP in area CA1 [30]. In agreement with our previous findings, the present results showed that the level of phosphorylated (p)-CREB in area CA1 was markedly reduced in Aβ rats (0.633 ± 0.075, $p = 0.05$). This reduction was completely prevented by long-term moderate treadmill exercise as Ex/Aβ rats had a similar level of p-CREB compared to those of control and exercise rats (control 1.102 ± 0.113, exercise 1.34 ± 0.093, Ex/Aβ 1.151 ± 0.176; Fig. 1a).

The levels of total (phosphorylated and un-phosphorylated) (t)-CREB in CA1 area among all groups were not different (control 1.896 ± 0.094, Aβ 1.962 ± 0.187, exercise 2.14 ± 0.328, Ex/Aβ 2.058 ± 0.107; Fig. 1b). Accordingly, the p-CREB:t-CREB ratio of Aβ rats in CA1 area was markedly lower than those of the control, exercise, and Ex/Aβ groups (control 0.624 ± 0.094, Aβ 0.3245 ± 0.0223, exercise 0.7533 ± 0.1285, Ex/Aβ 0.6447 ± 0.2212) ($p = 0.01$–0.05; Fig. 1c).

Similar results were seen in the DG area where long-term regular exercise prevented the decrease in the level of p-CREB. The level in DG area of Ex/Aβ rats was 1.542 ± 0.3476, which was not statistically different from that of control (1.332 ± 0.3161) and exercise (1.542 ± 0.1901) rats but significantly different from that of DG area of Aβ rats (0.899 ± 0.2753) ($p = 0.001$–0.05; Fig. 2a). Similar to area CA1, the level of t-CREB in the DG area was statistically unaltered in all groups (control 1.976 ± 0.5127, Aβ 1.856 ± 0.531, exercise 1.811 ± 0.4626, Ex/Aβ 1.949 ± 0.423) (Fig. 2b). Therefore, the p-CREB:t-CREB ratio in DG area of Aβ rats was significantly lower than those of the other groups (control 0.7949 ± 0.2318, Aβ 0.4454 ± 0.1443, exercise 0.912 ± 0.1323, Ex/Aβ 0.9257 ± 0.2681) ($p = 0.01$–0.05; Fig. 2c). These findings may suggest that Aβ specifically impaired the process of phosphorylation without affecting the synthesis of this molecule in both hippocampal areas and that regular exercise efficiently prevents deleterious effects of AD pathology.

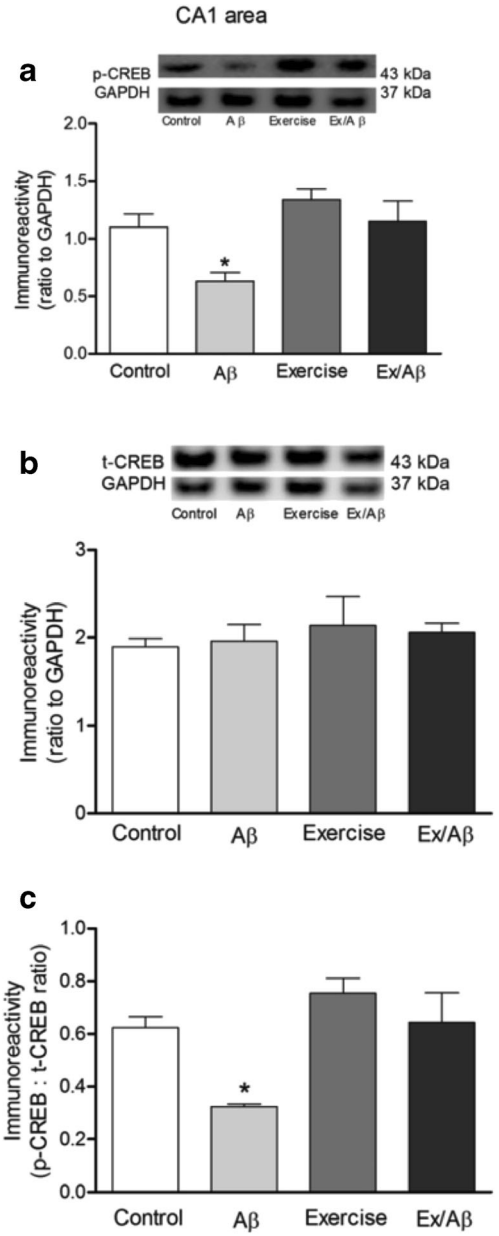

Fig. 1 Basal levels of p-CREB (**a**), t-CREB (**b**), and p-CREB: t-CREB ratio (**c**) in area CA1 of experimental groups. The basal levels of p-CREB in Aβ rats are significantly reduced compared to other groups while the levels of t-CREB are similar across all groups. The resulting p-CREB/t-CREB ratio of Aβ rats is significantly smaller than those of control, exercise, and Ex/Aβ rats, which indicates an impaired phosphorylation process. Regular treadmill exercise prevents amyloid infusion-induced reduction in basal levels of p-CREB and p-CREB/t-CREB ratio. *Indicates significant difference from control, exercise, and Ex/Aβ (one-way ANOVA; $p < 0.05$). Values are mean ± S.E.M., $n = 4$–6 rats/group. Insets are representative blots

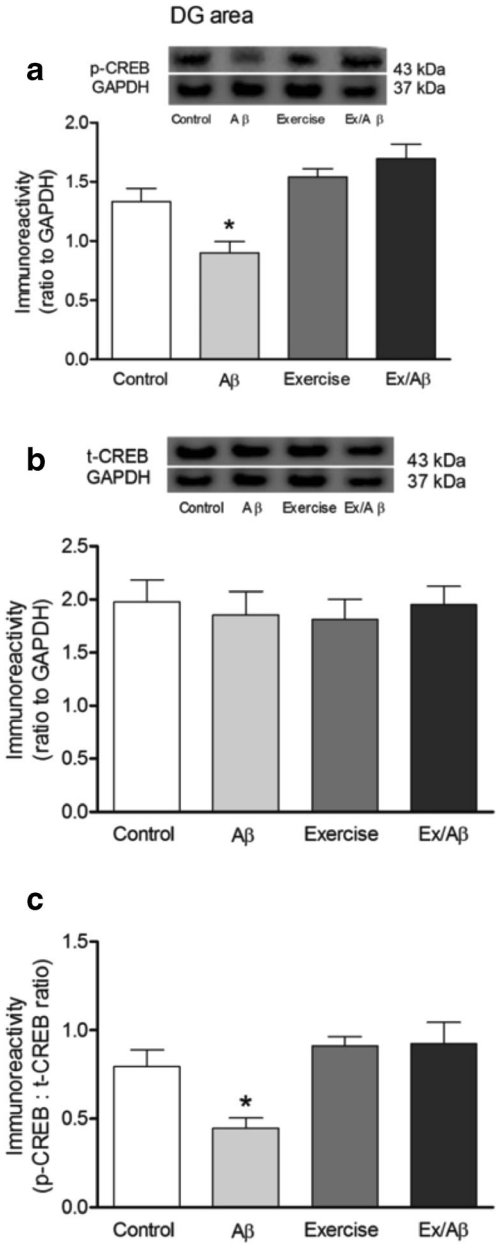

Fig. 2 Basal levels of p-CREB (**a**), t-CREB (**b**), and p-CREB:t-CREB ratio (**c**) in DG area. The basal levels of p-CREB in Aβ rats are significantly reduced compared to other groups while the levels of t-CREB are similar across all groups. Thus, the p-CREB/t-CREB ratio of Aβ rats is significantly smaller than those of control, exercise, and Ex/Aβ rats. Regular treadmill exercise prevents impairment of phosphorylation of p-CREB and reduction in p-CREB/t-CREB ratio caused by amyloid infusion. *Indicates significant difference from control, exercise, and Ex/Aβ ($p < 0.05$). Values are mean ± S.E.M., $n = 4$–6 rats/group. Insets are representative blots

Ca^{2+}/Calmodulin-Dependent Protein Kinase IV (*CaMKIV*)

CaMKIV is important for the CREB-related transcription process; it activates CREB through phosphorylation at Ser133 and has been associated with long-term memory consolidation and L-LTP expression [2, 31–33]. The present findings indicated that the level of CaMKIV (Aβ 0.4485 ± 0.0815) in area CA1 of Aβ rats was markedly reduced compared to the other groups and that regular exercise prevented this impairment (control 0.9216 ± 0.2001, exercise 0.9746 ± 0.1501, Ex/Aβ 1.068 ± 0.0712; $p = 0.001$) (Fig. 3a).

The level of CaMKIV in Aβ rats (0.2728 ± 0.0303) was also markedly reduced in the DG area ($p = 0.01$–0.05; Fig. 3b). This effect was prevented with regular exercise where the level of CaMKIV in the DG area of exercised Aβ rats (Ex/Aβ group: 0.6146 ± 0.1366) was not different from those of control (0.7075 ± 0.050) and exercise (0.6914 ± 0.1479) rats but significantly higher than that of Aβ rats.

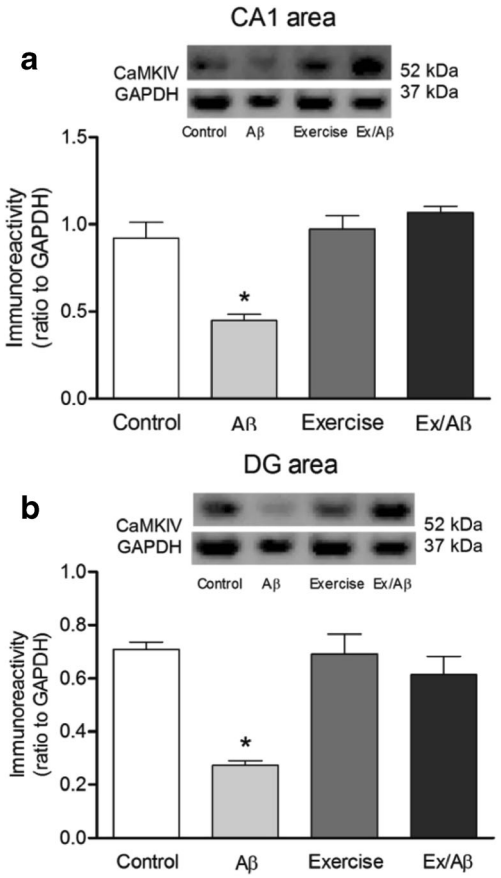

Fig. 3 **a** Western blot analysis of the basal levels of CaMKIV in the area CA1. Continuous amyloid infusion decreases the basal level of CaMKIV, and this reduction is prevented by treadmill exercise. The basal level of CaMKIV in Ex/Aβ rats is normal compared to those of control and exercise rats while this level is significantly lower in Aβ rats. *Indicates significant difference from control, exercise, and Ex/Aβ groups ($p < 0.05$). Values are mean ± S.E.M., $n = 4$–6 rats/group. Insets are representative blots. **b** Basal levels of CaMKIV in DG area. In our rat model of Alzheimer's disease, the basal level of CaMKIV is markedly reduced and this deleterious reduction is prevented by 4 weeks of treadmill exercise. The basal level of CaMKIV in Ex/Aβ rats is similar to those of control and exercise rats. *Indicates significant difference from control, exercise, and Ex/Aβ groups ($p < 0.05$). Values are mean ± S.E.M., $n = 4$–6 rats/group. Insets are representative blots

Phosphorylated and Total ERK1/2

The ERK signaling cascade is associated with the learning and memory processes [34]. To investigate the role of ERK1/2 signaling pathway in AD pathology, we measured the levels of both phosphorylated (p-) and total (t-) ERK1/2 in the hippocampal CA1 and DG areas. Surprisingly, our data revealed that in area CA1, the levels of p-ERK1/2 were statistically unchanged in all groups (control 1.0 ± 0.2659, Aβ 0.9245 ± 0.3049, exercise 1.145 ± 0.3389, Ex/Aβ 0.9538 ± 0.3393; Fig. 4a). Also, neither amyloid infusion nor exercise training altered the level of t-ERK1/2 in area CA1 as this level was similar among all groups (control 1.0 ± 0.204, Aβ 0.8983 ± 0.1512, exercise 1.086 ± 0.3048, Ex/Aβ 0.8713 ± 0.2212; Fig. 4b). As a result, the p-ERK1/2:t-ERK1/2 ratios of control (1.025 ± 0.3046), Aβ (1.086 ± 0.5007), exercise (0.9514 ± 0.3404), and Ex/Aβ (1.098 ± 0.29) were not significantly different (Fig. 4c).

Western blot analysis of total homogenates of the DG area revealed similar findings to those of area CA1. The p-ERK1/2 levels in the DG area for control, Aβ, exercise, and Ex/Aβ were 0.9675 ± 0.2414, 0.8819 ± 0.2531, 0.9661 ± 0.1503, and 0.9651 ± 0.1201 respectively (Fig. 5a). The total pool of ERK1/2 protein in DG area was also unaffected by amyloid infusion or treadmill exercise (control 1.0 ± 0.23, Aβ 0.9632 ± 0.2611, exercise 0.9503 ± 0.2178, Ex/Aβ 0.8446 ± 0.1157) (Fig. 5b). Thus, the p-ERK1/2:t-ERK1/2 ratios of all groups were not significantly different (control 1.027 ± 0.1432, Aβ 0.9004 ± 0.3077, exercise 0.9595 ± 0.1442, Ex/Aβ 1.037 ± 0.2296; Fig. 5c).

Discussion

Phosphorylated CREB is an important transcription factor responsible for local protein synthesis to produce long-lasting changes at the synapse [1] and is necessary for L-LTP maintenance. Examination of post-mortem AD brains, isolated hippocampal neurons of transgenic AD mice, and cultured neurons exposed to amyloid peptides has revealed that AD pathology negatively affects the protein expression of CREB [35, 36]. Consequently, downregulation of CREB results in reduction of downstream molecules, including neuronal glucose transporter 3 (GLUT3) culminating in decreased glucose uptake and metabolism in the AD brains [37]. In agreement with these findings, our present experiments showed a significant reduction of p-CREB levels in both CA1 and DG areas of Aβ rats. The decrease in p-CREB level of Aβ rats may be responsible for the long-term memory deficit [38] and L-LTP impairment in CA1 area of these rats [30].

One possible explanation of AD-induced decrease in p-CREB levels is negative modulation by the AD pathology of CREB upstream regulators such as ERK1/2 and CaMKIV.

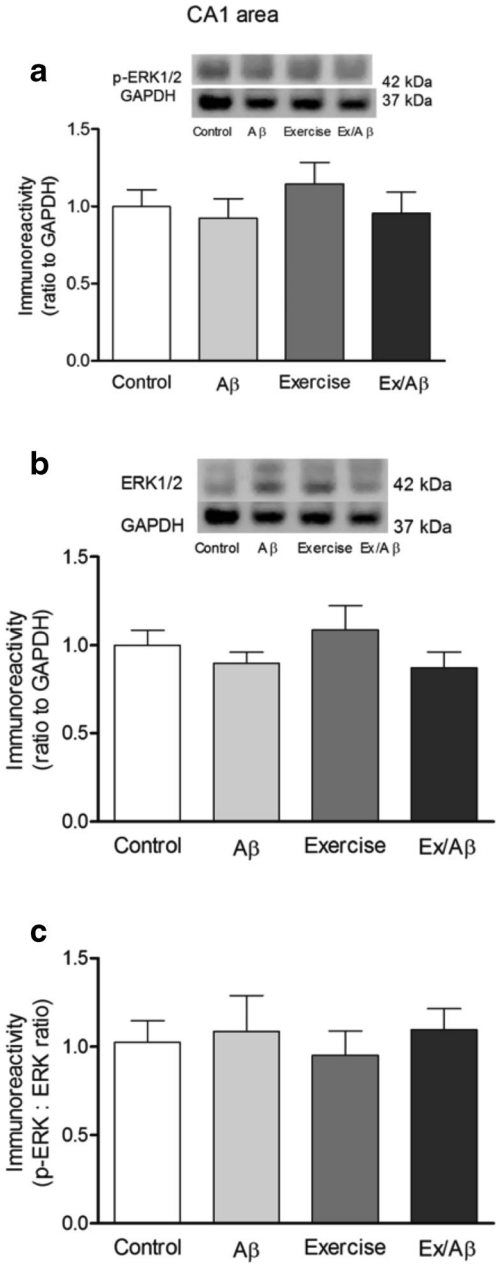

Fig. 4 Basal levels of p-ERK1/2 (**a**), t-ERK1/2 (**b**), and p-ERK1/2: t-ERK1/2 ratio (**c**) in area CA1 represented by the 42KDa fraction. There are no differences in the basal levels of p-ERK1/2, t-ERK1/2, and p-ERK1/2: t-ERK1/2 ratio across all groups. Values are mean ± S.E.M., $n = 4–6$ rats/group. Insets are representative blots

However, the present experiments ruled out the involvement of ERK1/2 in the downregulation of p-CREB inasmuch as we found no significant effect of Aβ on ERK1/2 levels. In contrast, regular treadmill exercise was efficient in preventing the impairment of the CREB/CaMKIV pathway associated with AD pathology. Neither exercise training nor amyloid infusion altered the levels of t-CREB in the CA1 and DG areas suggesting a functional impairment of CREB phosphorylation rather than its protein expression. This has been further confirmed by the markedly decreased levels of CaMKVI. Thus,

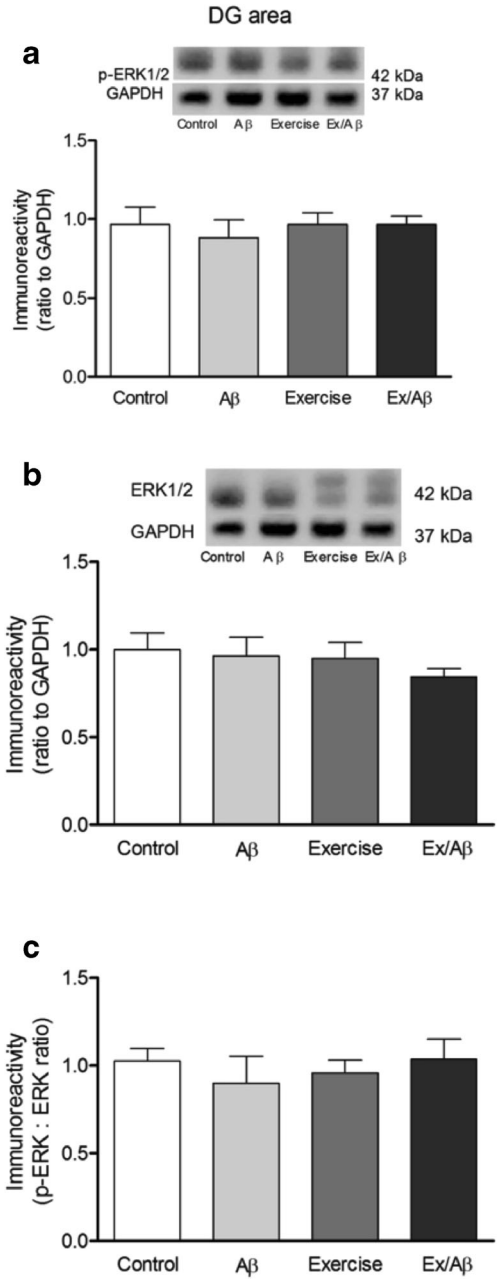

Fig. 5 Basal levels of p-ERK1/2 (**a**), t-ERK1/2 (**b**), and p-ERK1/2: t-ERK1/2 ratio (**c**) in DG area represented by the 42KDa fraction. There are no differences in the basal levels of p-ERK1/2, t-ERK1/2, and p-ERK1/2: t-ERK1/2 ratio across all groups. Values are mean ± S.E.M., $n = 4–6$ rats/group. Insets are representative blots

the downregulation of p-CREB but not total CREB suggests interference with the process of phosphorylation. This has been also confirmed by the markedly low p-CREB/t-CREB ratio in both CA1 and DG areas of Aβ rats.

In addition, our previous findings indicated that MHFS induced a robust increase of t-CREB, but not p-CREB, in both CA1 and DG areas of Aβ rats [38]. The deleterious effects of Aβ on the CREB pathway were totally prevented in regularly exercised Aβ-treated rats. An intact CREB has been reported to play a critical role in facilitating the positive effects of exercise-induced increase of BDNF, which eventually leads to better memory performance in exercised animals [39, 40]. We have shown exercise to have a profound positive ability to upregulate levels of BDNF even in brain of AD animal model [26, 27].

Parallel with the downregulation in the levels of p-CREB, we showed a similar decrease in levels of CaMKIV in CA1 and DG areas of Aβ rats. Surprisingly, however, we detected no significant changes in the levels of phosphorylated or total ERK1/2 in both hippocampal areas across all groups. Therefore, it may be that CaMKIV is the principal kinase for phosphorylation of CREB. In a study in double transgenic mice model of AD, which showed a reduced protein expression of CaMKIV, the authors suggested that by restricting CaMKIV availability, AD pathology reduced CREB phosphorylation, and consequently inhibited L-LTP and impaired spatial long-term memory [41]. The fact that 4 weeks of treadmill exercise was able to prevent AD-induced downregulation of CaMKIV levels indicated that exercise could sustain normal CREB phosphorylation thus maintaining an intact L-LTP even under the onslaught of $A\beta_{1–42}$ toxicity.

Interestingly, in normal animals, exercise by itself showed no significant effect on any of the signaling molecules studied in the present report or the variety of other signaling molecules studied previously. Additionally, exercise did not affect cognitive function or synaptic plasticity in normal animals. Interestingly, the only notable exception is the effect of exercise on BDNF molecule, where exercise invariably highly upregulated BDNF levels even in the presence of a disorder [25–27, 42–45]. Therefore, we conclude that exercise is an efficient neuroprotective agent possibly through upregulation of BDNF.

In the present study, we observed that neither exercise nor amyloid infusion or the combination thereof produced any changes in the levels of phosphorylated and total ERK1/2 in both CA1 and DG areas, among all groups. Contrary to these findings, some investigators have reported ERK1/2 signaling dysfunction specific to AD in fibroblasts [46] while voluntarily running can markedly upregulate the expression of ERK1/2 in exercised animals [47]. Others reported an increase in ERK p-38 in an Aβ rat model of AD [48, 49], which could be an inflammatory reaction to the introduction of Aβ into hippocampal tissue, as suggested by marked elevation of inflammatory cytokines. This incongruity could be due to variations of techniques employed in each study and the time at which the protein level was measured as exercise-induced increase in levels of p-ERK1/2 is often delayed [47].

Even though the CA1 and DG areas of the hippocampus share close physical proximity and display similar laminar arrangement and have similar LTP mechanisms, these two areas are entirely different with regard to cell type, resistance to insults, distributions, and regulations of neurotransmitters

and receptors among other differences [50–53]. Another important distinguishing feature of the DG area is neurogenesis, which takes place under both physiological and pathological conditions [54].

Although, in the present report, we found no differences between CA1 and DG areas related to these signaling molecules in our AD model, we have reported that infusion of Aβ severely blocked E-LTP and L-LTP represented by pspike in area CA1, [25], without affecting those of the DG area [26, 27]. The pspike amplitude describes firing of all neurons including new ones while the fEPSP slope represents only existing neurons with functional synapses in the circuit. The likely reason for resistance of the pspike of the DG areas to the onslaught of AD is the unique ability of this hippocampal area for neurogenesis. Therefore, since the pspike amplitude is a measure of the number of neurons reaching threshold for firing, it is possible that the DG produced enough new cells to preserve the integrity of the pspike.

References

1. Lonze BE, Ginty DD (2002) Function and regulation of CREB family transcription factors in the nervous system. Neuron 35: 605–623
2. Kang H, Sun LD, Atkins CM, Soderling TR, Wilson MA, Tonegawa S (2001) An important role of neural activity-dependent CaMKIV signaling in the consolidation of long-term memory. Cell 106:771–783
3. Chow FA, Anderson KA, Noeldner PK, Means AR (2005) The autonomous activity of calcium/calmodulin-dependent protein kinase IV is required for its role in transcription. J Biol Chem 280: 20530–20538
4. Ho N, Liauw JA, Blaeser F, Wei F, Hanissian S, Muglia LM, Wozniak DF, Nardi A et al (2000) Impaired synaptic plasticity and cAMP response element-binding protein activation in Ca2+/calmodulin-dependent protein kinase type IV/Gr-deficient mice. J Neurosci 20:6459–6472
5. Limbäck-Stokin K, Korzus E, Nagaoka-Yasuda R, Mayford M (2004) Nuclear calcium/calmodulin regulates memory consolidation. J Neurosci 24:10858–10867
6. Lee E, Son H (2009) Adult hippocampal neurogenesis and related neurotrophic factors. BMB Rep 42:239–244
7. Fukushima H, Maeda R, Suzuki R, Suzuki A, Nomoto M, Toyoda H, Wu LJ, Xu H et al (2008) Upregulation of calcium/calmodulin-dependent protein kinase IV improves memory formation and rescues memory loss with aging. J Neurosci 28:9910–9919
8. Pittenger C, Kandel E (1998) A genetic switch for long-term memory. C R Acad Sci III 321:91–96
9. Alberini CM (2009) Transcription factors in long-term memory and synaptic plasticity. Physiol Rev 89:121–145
10. Kida S (2012) A functional role for CREB as a positive regulator of memory formation and LTP. Exp Neurobiol 21:136–140
11. Shaywitz AJ, Greenberg ME (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem 68:821–861
12. Du K, Asahara H, Jhala US, Wagner BL, Montminy M (2000) Characterization of a CREB gain-of-function mutant with constitutive transcriptional activity in vivo. Mol Cell Biol 20:4320–4327
13. Barco A, Patterson SL, Alarcon JM, Gromova P, Mata-Roig M, Morozov A, Kandel ER (2005) Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. Neuron 48:123–137
14. Fan N, Yang H, Zhang J, Chen C (2010) Reduced expression of glutamate receptors and phosphorylation of CREB are responsible for in vivo Delta9-THC exposure-impaired hippocampal synaptic plasticity. J Neurochem 112:691–702
15. Middei S, Houeland G, Cavallucci V, Ammassari-Teule M, D'Amelio M, Marie H (2013) CREB is necessary for synaptic maintenance and learning-induced changes of the ampa receptor GluA1 subunit. Hippocampus 23:488–499
16. Jancic D, Lopez de Armentia M, Valor LM, Olivares R, Barco A (2009) Inhibition of cAMP response element-binding protein reduces neuronal excitability and plasticity, and triggers neurodegeneration. Cereb Cortex 19:2535–2547
17. Giovannini MG (2006) The role of the extracellular signal-regulated kinase pathway in memory encoding. Rev Neurosci 17: 619–634
18. Xia Z, Storm DR (2012) Role of signal transduction crosstalk between adenylyl ncyclase and MAP kinase in hippocampus-dependent memory. Learn Mem 19:369–374
19. Kamei H, Nagai T, Nakano H, Togan Y, Takayanagi M, Takahashi K, Kobayashi K, Yoshida S et al (2006) Repeated methamphetamine treatment impairs recognition memory through a failure of novelty-induced ERK1/2 activation in the prefrontal cortex of mice. Biol Psychiatry 59:75–84
20. Huang CH, Chiang YW, Liang KC, Thompson RF, Liu IY (2010) Extra-cellular signal-regulated kinase 1/2 (ERK1/2) activated in the hippocampal CA1 neurons is critical for retrieval of auditory trace fear memory. Br Res 1326:143–151
21. Roskoski R Jr (2012) ERK1/2 MAP kinases: structure, function, and regulation. Pharmacol Res 66:105–143
22. Mao L, Tang Q, Samdani S, Liu Z, Wang JQ (2004) Regulation of MAPK/ERK phosphorylation via ionotropic glutamate receptors in cultured rat striatal neurons. Eur J Neurosci 19:1207–1216
23. Tiraboschi E, Tardito D, Kasahara J, Moraschi S, Pruneri P, Gennarelli M, Racagni G, Popoli M (2004) Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. Neuropsychopharmacology 29:1831–1840
24. English JD, Sweatt JD (1997) A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. J Biol Chem 272:19103–19106
25. Dao AT, Zagaar MA, Levine AT, Salim S, Eriksen JL, Alkadhi KA (2013) Treadmill exercise prevents learning and memory impairment in Alzheimer's disease-like pathology. Curr Alzheimer Res 10:507–515
26. Dao AT, Zagaar MA, Salim S, Eriksen JL, Alkadhi KA (2014) Regular exercise prevents non-cognitive disturbances in a rat model of Alzheimer's disease. Int J Neuropsychopharmacol 17:593–602
27. Dao AT, Zagaar MA, Alkadhi KA (2015) Moderate treadmill exercise protects synaptic plasticity of the dentate gyrus and related signaling cascade in a rat model of Alzheimer's disease. Mol Neurobiol 52:1067–1076
28. Kandel ER (2012) The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. Mol Brain 5:14
29. Ran I, Laplante I, Lacaille JC (2012) CREB-dependent transcriptional control and quantal changes in persistent long-term potentiation in hippocampal interneurons. J Neurosci 32:6335–6350
30. Alkadhi KA, Alzoubi KH, Srivareerat M, Tran TT (2011) Chronic psychosocial stress exacerbates impairment of synaptic plasticity in β-amyloid rat model of Alzheimer's disease: prevention by nicotine. Curr Alzheimer Res 8:718–731

31. Miyamoto E (2006) Molecular mechanism of neuronal plasticity: induction and maintenance of long-term potentiation in the hippocampus. J Pharmacol Sci 100:433–442
32. Kasahara J, Fukunaga K, Miyamoto E (2001) Activation of calcium/calmodulin-dependent protein kinase IV in long term potentiation in the rat hippocampal CA1 region. J Biol Chem 276: 24044–24050
33. Smolen P, Baxter DA, Byrne JH (2006) A model of the roles of essential kinases in the induction and expression of late long-term potentiation. Biophys J 90:2760–2775
34. Peng S, Zhang Y, Zhang J, Wang H, Ren B (2010) ERK in learning and memory: a review of recent research. Int JMol Sci 11:222–232
35. Pugazhenthi S, Wang M, Pham S, Sze CI, Eckman CB (2011) Downregulation of CREB expression in Alzheimer's brain and in Abeta-treated rat hippocampal neurons. Mol Neurodegener 6:60
36. Ljungberg MC, Ali YO, Zhu J, Wu CS, Oka K, Zhai RG, Lu HC (2012) CREB-activity and nmnat2 transcription are down-regulated prior to neurodegeneration, while NMNAT2 over-expression is neuroprotective, in a mouse model of human tauopathy. Human Mol Gen 21:251–267
37. Jin N, Qian W, Yin X, Zhang L, Iqbal K, Grundke-Iqbal I, Gong CX, Liu F (2013) CREB regulates the expression of neuronal glucose transporter 3: a possible mechanism related to impaired brain glucose uptake in Alzheimer's disease. Nucleic Acids Res 41: 3240–3256
38. Dao AT, Zagaar MA, Levine AT, Alkadhi KA (2016) Comparison of the effect of exercise on late-phase LTP of the dentate gyrus and CA1 of Alzheimer's disease model. Mol Neurobiol 53:6859–6868
39. Vaynman S, Ying Z, Gomez-Pinilla F (2003) Interplay between brain-derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. Neuroscience 122:647–657
40. Chen MJ, Russo-Neustadt AA (2009) Running exercise-induced up-regulation of hippocampal brain-derived neurotrophic factor is CREB-dependent. Hippocampus 19:962–972
41. Arrazola MS, Varela-Nallar L, Colombres M, Toledo EM, Cruzat F, Pavez L, Assar R, Aravena A et al (2009) Calcium/calmodulin-dependent protein kinase type IV is a target gene of the Wnt/beta-catenin signaling pathway. J Cell Physiol 221:658–667
42. Zagaar M, Dao A, Levine A, Alhaider I, Alkadhi K (2013) Regular exercise prevents sleep deprivation associated impairment of long-term memory and synaptic plasticity in the CA1 area of the hippocampus. Sleep 36:751–761
43. Zagaar M, Dao A, Alhaider I, Alkadhi K (2013) Regular treadmill exercise prevents sleep deprivation-induced disruption of synaptic plasticity and associated signaling cascade in the dentate gyrus. Mol Cell Neurosci 56:375–383
44. Cassilhas RC, Lee KS, Fernandes J, Oliveira MG, Tufik S, Meeusen R, de Mello MT (2012) Spatial memory is improved by aerobic and resistance exercise through divergent molecular mechanisms. Neuroscience 202:309–317
45. Bechara RG, Kelly AM (2013) Exercise improves object recognition memory and induces BDNF expression and cell proliferation in cognitively enriched rats. Behav Br Res 245:96–100
46. Zhao WQ, Ravindranath L, Mohamed AS, Zohar O, Chen GH, Lyketsos CG, Etcheberrigaray R, Alkon DL (2002) MAP kinase signaling cascade dysfunction specific to Alzheimer's disease in fibroblasts. Neurobiol Dis 11:156–163
47. Shen J, Maruyama IN (2012) Brain-derived neurotrophic factor receptor TrkB exists as a preformed dimer in living cells. J Mol Signal 7:2
48. Hu Q, Yu B, Chen Q, Wang Y, Ling Y, Sun S, Shi Y, Zhou C (2018) Effect of Linguizhugan decoction on neuroinflammation and expression disorder of the amyloid β-related transporters RAGE and LRP-1 in a rat model of Alzheimer's disease. Mol Med Rep 17: 827–834
49. Lv C, Wang L, Liu X, Yan S, Yan SS, Wang Y, Zhang W (2015) Multi-faced neuroprotective effects of geniposide depending on the RAGE-mediated signaling in an Alzheimer mouse model. Neuropharmacology 89:175–184
50. Hsu JC, Zhang Y, Takagi N, Gurd JW, Wallace MC, Zhang L, Eubanks JH (1998) Decreased expression and functionality of NMDA receptor complexes persist in the CA1, but not in the dentate gyrus after transient cerebral ischemia. J Cereb Blood Flow Metab 18:768–775
51. Yao H, Huang YH, Liu ZW, Wan Q, Ding AS, Zhao B, Fan M, Wang FZ (1998) The different responses to anoxia in cultured CA1 and DG neurons from newborn rats. Acta Phys Sin 50:61–66
52. Gerges NZ, Alkadhi KA (2004) Hypothyroidism impairs late LTP in CA1 region but not in dentate gyrus of the intact rat hippocampus: MAPK involvement. Hippocampus 14:40–45
53. Alzoubi KH, Aleisa AM, Alkadhi KA (2005) Impairment of long-term potentiation in the CA1, but not dentate gyrus, of the hippocampus in obese Zucker rats: role of calcineurin and phosphorylated CaMKII. J Mol Neurosci 27:337–346
54. Sawada M, Sawamoto K (2013) Mechanisms of neurogenesis in the normal and injured adult brain. Keio J Med 62:13–28