

Original Article



Treadmill exercise improves memory and increases hippocampal BDNF in a rat model of Alzheimer's Disease

Rokhsareh Abshenas^{1,2}, Tayebe Artimani^{1,2}, Iraj Amiri^{1,2}, Siamak Shahidi³, Sara Soleimani Asl^{1,2*} 

1. Endometrium and Endometriosis Research Centre, Hamadan University of Medical Sciences, Hamadan, Iran
2. Anatomy Department, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
3. Physiology Department, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Abstract

Introduction: Alzheimer's disease is strongly correlated with learning and memory impairments. As exercise can enhance memory and learning, in this study, we have investigated the effects of treadmill exercise on memory impairment in amyloid β ($A\beta$)-treated rats focusing on brain-derived neurotrophic factor (BDNF) expression.

Methods: Wistar male rats received intracerebroventricular (ICV) injection of $A\beta$ and exercised on a treadmill for one month. Memory function was assessed using Morris water maze (MWM) and avoidance learning tasks. The level of BDNF was examined by the ELISA test.

Results: The results of MWM and avoidance learning tasks showed that treadmill exercise could improve $A\beta$ -induced memory impairment significantly. Moreover, BDNF expression increased following exercise in the $A\beta$ -treated rats.

Conclusion: The present results suggested that treadmill exercise may improve memory in Alzheimer's disease by increasing BDNF level in the hippocampus.

<http://dx.doi.org/10.32598/ppj.24.4.50>

Keywords:

Treadmill exercise;
Alzheimer's disease;
Memory;
BDNF

* Corresponding author:

S. Soleimani Asl

Email:

s.soleimanasl@umsha.ac.ir

Tel: +98 (81) 38380208

Received 10 November 2019;

Received in revised form 11

June 2020; Accepted 15 June

2020

Introduction

Alzheimer's disease (AD) the most common cognitive disorders of the elderly is characterized by senile plaques of amyloid β ($A\beta$) and neurofibrillary tangles (Selkoe, 1997). The deposition of fibrillary $A\beta$ is strongly accepted as an agent in the pathogenesis of AD and associated with reduced cognition, increased reactive oxygen species and decreased cell count in the hippocampus (De Felice et al., 2007). It has been reported that up-regulation of BDNF leads to a decrease in $A\beta$ -induced neurotoxicity (Doi et al.,

2013). Cognition and memory functions are positively affected by brain-derived neurotrophic factor (BDNF) (Kim and Kim, 2013). BDNF is a member of the neurotrophin family of growth factors, which has been expressed in the highest level in the brain especially in the hippocampus and facilitates the release of glutamate and enhances the phosphorylation of the NR1 and NR2B subunits of the NMDA-receptor complex (Tyler and Pozzo-Miller, 2001). BDNF pathway is mediated by TrkB and p75 receptors that trigger the phosphatidylinositol 3 kinase,

phospholipase C gamma and intracellular signal-regulated kinase 1/2 signaling cascades (for more detail see a review by Bekinschtein et al.) (Bekinschtein et al., 2008).

Treadmill exercise has been accepted as a therapeutic strategy that induces protection in the brain of both human and rodents (Gharebaghi et al., 2017; Otsuka et al., 2016). Several studies suggest that exercise improves learning and memory function through an increase in the levels of BDNF (Jeon and Ha, 2017). Furthermore, treadmill exercise enhances neurogenesis and myelin repairment via the Wnt3/ β catenin signaling pathway and induces an increase in the expression of BDNF and myelin basic protein (Cheng et al., 2020).

In the present study, we hypothesized that treadmill exercise might improve A β - induced memory impairment through an increase in BDNF in the hippocampus of rats.

Materials and methods

Animals

Thirty-five adult male Wistar rats (250-300g) were obtained from the animal facility of Hamadan University of Medical Sciences (HUMS) and maintained under standard laboratory condition (12 h/h light/dark cycle, 20 \pm 2°C and 50% relative humidity) with free access to food and water. All experiments approved by the Ethical committee of HUMS (No: IR.UMSHA.AC.REC.1396.99). The rats randomly classified into four groups (n=7 per each group): control, sham-operated, A β and A β + exercise groups.

Injection of A β

To induce AD, we performed intracerebroventricular (ICV) injection of A β (1-42, Sigma-Aldrich, St Louis, MO, USA) according to the previously described method (Komaki et al., 2019). Briefly, anesthetized rats were placed in a stereotaxic frame and the skull drilled over the lateral ventricle using the following coordinate: AP: -0.9mm from the bregma; ML: 1.6mm from the midline; DV: 2.0 mm from the skull surface (Paxinos and Watson, 2006) and A β (5 μ g/5 μ l) was injected slowly. The sham-operated group went under surgery similarly to the A β group except for A β injection.

A β peptides (1-42) represent very different conformational states so that the residues 31-34 and

38-41 form a β -hairpin, which causes a reduction in the flexibility of C-terminal and the greater propensity of A β 42 to form amyloids (Chen et al., 2017).

Treadmill exercise protocol

The day after the injection of A β , we used a motorized rodent treadmill instrument (Tajhiz Gostare Omide Iranian, Iran) to exercise according to a previously published protocol (Gharebaghi et al., 2017). The rats ran on the treadmill for one month (30min daily and five constitutive days/week). The exercise consisted of running at the speed of 25m/min with a 0.3mA stimulus current electric shock when the rats entered the rear of the test chamber.

Morris water maze (MWM) task

Spatial memory was assessed (Gharebaghi et al., 2017) using water filled MWM (210 \times 51cm), the day after the last day of ran on the last day of ran on the treadmill. There was a hidden platform located at a fixed position in the pool. Four consecutive training days consisting of two-block with four trials were conducted. In each trial, the rats were allowed to swim in the pool for 60min at different points. There were 30s and 5min inter-trial and inter-block intervals, respectively. Escape latency and the distance to reach (traveled distance) the hidden platform were recorded as the parameter of acquisition memory. Using a camera located above the center of the maze. On day 5, the platform was removed and the percentage of time spent in the target quadrant assessed as a parameter of the retention of memory.

Inhibitory avoidance apparatus (Shuttle-box)

The shuttle box apparatus consisted of two compartments (white and black) separated with a guillotine door. There was a stainless steel shock grid floor in the dark compartment. The day after the spatial memory assessment by MWM, the rats were placed in the white chamber and after 5s the guillotine door was opened and the rats entered the dark compartment. The door was closed and the rats received an electronic foot shock (50Hz and 1.5mA intensity) for 3s. After 24h, the time in the dark compartment (TDC) and step through latency (STL) were recorded to evaluate the avoidance learning memory. The latency was recorded a maximum of 300s.

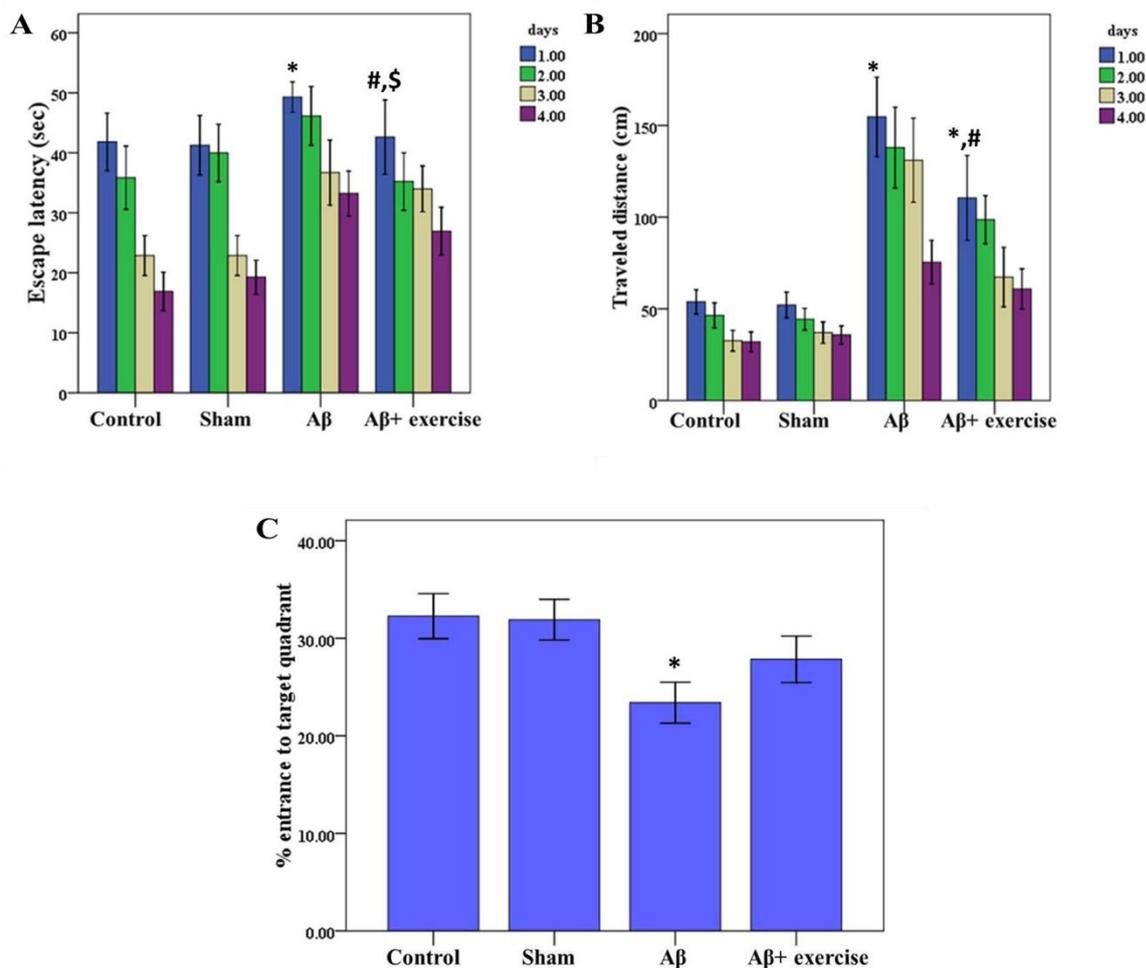


Fig.1. Protective effects of treadmill exercise in A β (1-42)-induced Alzheimer's model in the water-maze test. Each block signifies the mean of latencies (A) and traveled distance (B) to reach the hidden platform during four consecutive trial days in the MWM. A: * P <0.001 vs. control and sham groups; # P <0.05 vs. control and sham groups; \$ P <0.01 vs. A β group. B: * P <0.001 vs. control and sham groups; # P <0.001 vs. A β group. C: Represents the mean of the percentage of the entrance to the target quarter in the probe trial in the MWM, * P <0.05 vs. control group. Each value is the mean \pm SEM.

Enzyme-linked immunosorbent assay (ELISA)

Three hippocampi from each group were mixed and sonicated in PBS. BDNF level was quantified using an ELISA kit (ZellBio, Ulm, Germany) according to the manufactures recommendation. Assays were carried out in duplicate trials.

Statistical analysis

Statistical analysis was performed using SPSS 16. The repeated measure, one- way analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to analyze the significance between the groups. P -value <0.05 was considered significant.

Results

MWM performance

To assess acquisition memory, escape latency and traveled distance of four constitutive days were

analyzed using two-way ANOVA test with treatment as one factor and training days as the second factor. The results of escape latency showed a significant effect for training days [F(3, 2049)= 81.85, P <0.001] and treatment [F(4, 44.03)= 15.52, P <0.001]. There was a significant interaction between training days and treatment [F(12,538)= 2.14, P <0.05]. Further analysis indicated that A β - treated rats took more time to reach the hidden platform than control and sham-operated groups (P <0.001, Fig. 1A). According to the results, treadmill exercise for one month caused a significant reduction in escape latency in comparison to the A β group (P <0.01). The results of traveled distance showed a significant effect for training days [F(3, 5890)= 53.68, P <0.001] and treatment [F(4, 3032)= 276.4, P <0.001]. There was a significant interaction between treatment and training days [F(12, 7844)= 7.15, P <0.001]. One-way ANOVA

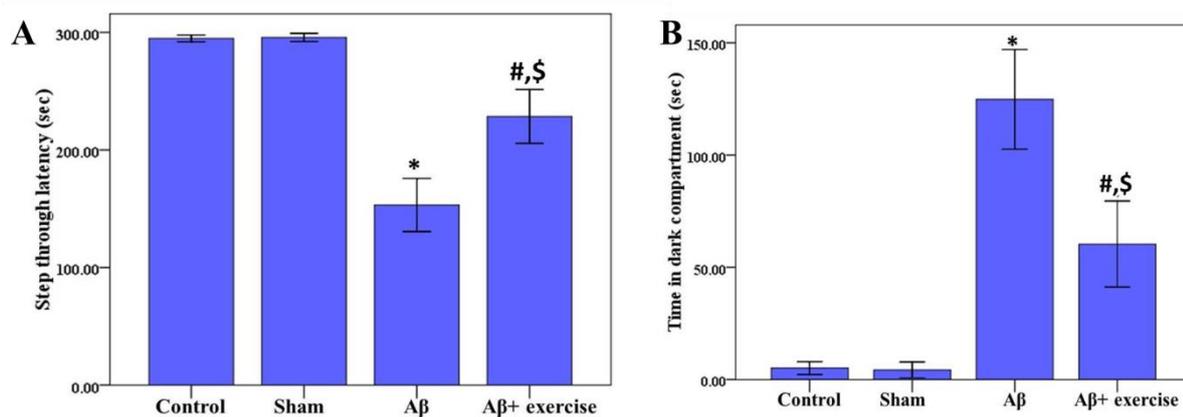


Fig.2. Protective effects of treadmill exercise in Aβ (1-42)-induced Alzheimer's model in the Shuttle box apparatus. The mean of step-through latency (A) and time spent in the dark compartment (B) in the passive avoidance task. A: * $P < 0.001$ vs. control and sham groups; # $P < 0.05$ vs. control and sham groups; \$ $P < 0.05$ vs. Aβ group. B: * $P < 0.001$ vs. control and sham groups; # $P < 0.05$ vs. control and sham groups; \$ $P < 0.05$ vs. Aβ group. Each value is the mean \pm SEM.

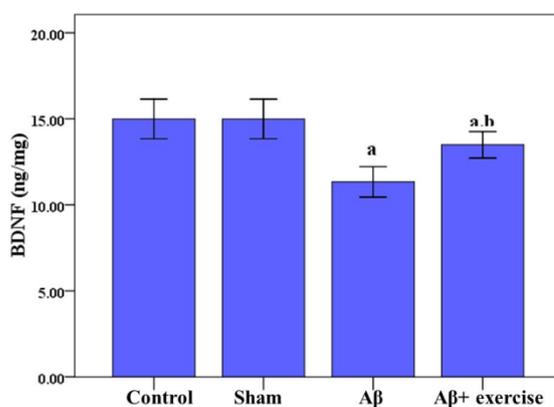


Fig.3. The effects of Aβ and treadmill exercise on the BDNF concentration of hippocampus homogenate samples. Mean value (\pm SEM) of BDNF level in the hippocampus. * $P < 0.001$ vs. control and sham groups; # $P < 0.05$ vs. Aβ group.

analysis of training days revealed that Aβ-treated group swam further to reach the platform, which was significant when compared to the control and sham-operated groups ($P < 0.001$, Fig. 1B). Treadmill exercise decreased traveled distance compared with Aβ group ($P < 0.001$).

In the probe trial session, we found a significant difference between Aβ-treated rats and the control group ($P < 0.05$, Fig. 1C) and Aβ group spent less time in the target quadrant. Treadmill exercise caused an increase in the entrance to the target quadrant (27.85 ± 2.37) compared with Aβ-treated rats (23.39 ± 2.10) without any significant difference.

Passive avoidance task

Our results revealed a significant difference between control, sham and Aβ groups ($P < 0.001$, Fig. 2A) in STL. Aβ-treated rats that exercised for one month showed a significant increase in STL concerning the

Aβ group ($P < 0.01$). Furthermore, control and sham-operated groups spent less time in the dark compartment compared to the Aβ group ($P < 0.001$, Fig. 2B). Treadmill exercise significantly attenuated TDC when compared to the Aβ group ($P < 0.01$).

ELISA for BDNF

As shown in Figure 3, the ICV injection of Aβ led to a significant reduction in the BDNF level in the hippocampus compared with the control and sham group ($P < 0.001$). We found a significant increase in BDNF level in rats undergoing exercise than Aβ-treated rats ($P < 0.05$).

Discussion

Learning and memory impairments are the important symptoms of AD and ICV injection of Aβ resulted in the structural and physiological alterations in the hippocampus, which contribute to cognitive deficit

(Leuner et al., 2012; van der Zee et al., 2008). In this study, we found an attenuation of learning and memory impairment by treadmill exercise in A β -induced AD model. Second, we showed that exercise could improve impaired memory through increased expression of BDNF in the hippocampus.

Previous studies have found a robust relation between physical exercise and cognition. They reported that exercise increases life span and prevents the decline of behavioral performance in middle age (Navarro et al., 2004) and the elderly (Kim et al., 2010). Treadmill exercise increased latency of the step-down avoidance and also decreased the latency and distance in MWM in the A β -injected rats, indicating that exercise attenuates A β -induced memory impairment. Our results supported finding by Kim et al. (2010) who reported that treadmill exercise is a useful strategy for preventing failure of memory in the elderly.

Physical exercise has also been shown to enhance cognitive function in a rat model of vascular dementia (Choi et al., 2016) and Alzheimer's disease (Koo et al., 2017). They concluded that treadmill exercise improves cognition deficits, possibly by increasing disintegrin and metalloproteinase domain-containing protein 10. In another study, latency in the passive avoidance test increased in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)/probenecid-induced Parkinson's disease in the mice model undergoing exercise (Sung, 2015). They reported an increased BDNF expression in the ventral midbrain of exercised animals and suggested that exercise can overcome Parkinson-induced memory impairment through enhancement of BDNF expression and prevention of dopaminergic neuronal damage.

BDNF as a member of the neurotrophic factors family plays an important role in cognition and memory function, neuronal survival and differentiation (Pang and Lu, 2004). It has been reported that exogenous application of BDNF improved hippocampal long-term potentiation impairment in BDNF knockout mice (Patterson et al., 1996). In the present study, the ICV injection of A β caused a reduction in the expression of BDNF in the hippocampus and a significant increase observed in BDNF expression in rats that underwent treadmill exercise. Consistent with our results, Molteni et al. (2002) suggested that exercise increased the expression of plasticity-related genes such as BDNF in the rat hippocampus.

In another study, exercise induced the synaptic plasticity markers through a BDNF-mediated mechanism in the hippocampus (Vaynman et al., 2003). Previously we found that treadmill exercise for one month alleviated cognitive deficit most likely by an increase in the BDNF expression (Sajadi et al., 2017). BDNF regulates neurogenesis, axonal and dendritic branching, and remodeling, as well as functional maturation of excitatory and inhibitory synapse (Seil and Drake-Baumann, 2000; Vicario-Abejón et al., 1998). Several studies have established a positive correlation between BDNF expression and the memory function (Slipczuk et al., 2009). They showed over-expression of BDNF increased neurogenesis in the hippocampus and improved spatial memory (Rossi et al., 2006). We found a significant increase in BDNF expression following exercise that involved long-term plasticity and memory. Based on the combined findings of this study, it can be inferred that treadmill exercise protects against memory impairment in AD model through an increase in BDNF expression.

Conclusion

In this study, avoidance learning and spatial memory in the A β -injected rats that underwent exercise were much better than those in the AD model. Our study revealed that BDNF expression was enhanced following exercise in the hippocampus. Therefore, it was concluded that treadmill exercise could be an important clinical strategy for preventing failure of memory in those with neurodegenerative disease.

Acknowledgments

This study was supported by the Hamadan University of Medical Sciences grant (9603201344).

Conflict of interest

The authors report no conflict of interest.

References

- Bekinschtein P, Cammarota M, Izquierdo I, Medina JH. BDNF and memory formation and storage. *Neuroscientist* 2008; 14: 147-56. <https://doi.org/10.1177/1073858407305850>
- Chen GF, Xu TH, Yan Y, Zhou YR, Jiang Y, Melcher K, et al. Amyloid beta: structure, biology and structure-based therapeutic development. *Acta Pharmacol Sin* 2017; 38: 1205-35. <https://doi.org/10.1038/aps.2017.28>
- Cheng J, Shen W, Jin L, Pan J, Zhou Y, Pan G, et al.

- Treadmill exercise promotes neurogenesis and myelin repair via upregulating Wnt/ β -catenin signaling pathways in the juvenile brain following focal cerebral ischemia/reperfusion. *Int J Mol Med* 2020; 45: 1447-63. <https://doi.org/10.3892/ijmm.2020.4515>
- Choi DH, Lee KH, Lee J. Effect of exercise-induced neurogenesis on cognitive function deficit in a rat model of vascular dementia. *Mol Med Rep* 2016; 13: 2981-90. <https://doi.org/10.3892/mmr.2016.4891>
- De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, et al. Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. *J Biol Chem* 2007; 282: 11590-601. <https://doi.org/10.1074/jbc.M607483200>
- Doi Y, Takeuchi H, Horiuchi H, Hanyu T, Kawanokuchi J, Jin S, et al. Fingolimod phosphate attenuates oligomeric amyloid β -Induced neurotoxicity via increased brain-derived neurotrophic factor expression in neurons. *PLoS One* 2013; 8: e61988. <https://doi.org/10.1371/journal.pone.0061988>
- Gharebaghi A, Amiri I, Salehi I, Shahidi S, Komaki A, Mehdizadeh M, et al. Treadmill exercise attenuates 3,4-methylenedioxymethamphetamine-induced memory impairment through a decrease apoptosis in male rat hippocampus. *J Neurosci Res* 2017; 95: 2448-55. <https://doi.org/10.1002/jnr.24078>
- Jeon YK, Ha CH. The effect of exercise intensity on brain derived neurotrophic factor and memory in adolescents. *Environ Health Prev Med* 2017; 22: 1-6. <https://doi.org/10.1186/s12199-017-0643-6>
- Kim G, Kim E. The effects of antecedent exercise on motor function recovery and brain-derived neurotrophic factor expression after focal cerebral ischemia in rats. *J Phys Ther Sci* 2013; 25: 553-6. https://doi.org/10.1589/jpts.25.5_553
- Kim SE, Ko IG, Kim BK, Shin MS, Cho S, Kim CJ, et al. Treadmill exercise prevents aging-induced failure of memory through an increase in neurogenesis and suppression of apoptosis in rat hippocampus. *Exp Gerontol* 2010; 45: 357-65. <https://doi.org/10.1016/j.exger.2010.02.005>
- Komaki H, Faraji N, Komaki A, Shahidi S, Etaee F, Raoufi S, et al. Investigation of protective effects of coenzyme Q10 on impaired synaptic plasticity in a male rat model of Alzheimer's disease. *Brain Res Bull* 2019; 147: 14-21. <https://doi.org/10.1016/j.brainresbull.2019.01.025>
- Koo JH, Kang EB, Oh YS, Yang DS, Cho JY. Treadmill exercise decreases amyloid β burden possibly via activation of SIRT-1 signaling in a mouse model of Alzheimer's disease. *Exp Neurol* 2017; 288: 142-52. <https://doi.org/10.1016/j.expneurol.2016.11.014>
- Leuner K, Müller WE, Reichert AS. From mitochondrial dysfunction to amyloid beta formation: novel insights into the pathogenesis of Alzheimer's disease. *Mol Neurobiol* 2012; 46: 186-93. <https://doi.org/10.1007/s12035-012-8307-4>
- Molteni R, Ying Z, Gómez -Pinilla F. Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. *Eur J Neurosci* 2002; 16: 1107-16. <https://doi.org/10.1046/j.1460-9568.2002.02158.x>
- Navarro A, Gomez C, López-Cepero JM, Boveris A. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. *Am J Physiol Regul Integr Comp Physiol* 2004; 286: R505-11. <https://doi.org/10.1152/ajpregu.00208.2003>
- Otsuka S, Sakakima H, Sumizono M, Takada S, Terashi T, Yoshida Y. The neuroprotective effects of preconditioning exercise on brain damage and neurotrophic factors after focal brain ischemia in rats. *Behav Brain Res* 2016; 303: 9-18. <https://doi.org/10.1016/j.bbr.2016.01.049>
- Pang PT, Lu B. Regulation of late-phase LTP and long-term memory in normal and aging hippocampus: role of secreted proteins tPA and BDNF. *Ageing Res Rev* 2004; 3: 407-30. <https://doi.org/10.1016/j.arr.2004.07.002>
- Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron* 1996; 16: 1137-45. [https://doi.org/10.1016/S0896-6273\(00\)80140-3](https://doi.org/10.1016/S0896-6273(00)80140-3)
- Paxinos G, Watson C. The rat brain in stereotaxic coordinates: hard cover edition. Academic Press 2006.
- Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, Babbini F, et al. Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *Eur J Neurosci* 2006; 24: 1850-6. <https://doi.org/10.1111/j.1460-9568.2006.05059.x>
- Sajadi A, Amiri I, Gharebaghi A, Komaki A, Asadbegi M, Shahidi S, et al. Treadmill exercise alters ecstasy-induced long-term potentiation disruption in the hippocampus of male rats. *Metab Brain Dis* 2017; 32: 1603-7. <https://doi.org/10.1007/s11011-017-0046-9>
- Seil FJ, Drake-Baumann R. Neurotrophins and activity-dependent inhibitory synaptogenesis. *Prog Brain Res* 2000; 128: 219-229. [https://doi.org/10.1016/S0079-6123\(00\)28019-9](https://doi.org/10.1016/S0079-6123(00)28019-9)
- Selkoe DJ. Alzheimer's disease: genotypes, phenotypes, and treatments. *Science* 1997; 275: 630-1. <https://doi.org/10.1126/science.275.5300.630>
- Slipczuk L, Bekinschtein P, Katche C, Cammarota M, Izquierdo I, Medina JH. BDNF activates mTOR to regulate GluR1 expression required for memory formation. *PLoS One* 2009; 4: e6007. <https://doi.org/10.1371/journal.pone.0006007>
- Sung YH. Effects of treadmill exercise on hippocampal neurogenesis in an MPTP /probenecid-induced Parkinson's disease mouse model. *J Phys Ther Sci* 2015; 27: 3203-6. <https://doi.org/10.1589/jpts.27.3203>
- Tyler WJ, Pozzo-Miller LD. BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses. *J Neurosci* 2001; 21: 4249-58.

- <https://doi.org/10.1523/JNEUROSCI.21-12-04249.2001>
van der Zee J, Slegers K, Van Broeckhoven C. Invited article: the Alzheimer disease-frontotemporal lobar degeneration spectrum. *Neurology* 2008; 71: 1191-7. <https://doi.org/10.1212/01.wnl.0000327523.52537.86>
- Vaynman S, Ying Z, Gomez-Pinilla F. Interplay between brain-derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. *Neuroscience* 2003; 122: 647-57. <https://doi.org/10.1016/j.neuroscience.2003.08.001>
- Vicario- Abejón C, Collin C, McKay RD, Segal M. Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons. *J Neurosci* 1998; 15; 18: 7256-71. <https://doi.org/10.1523/JNEUROSCI.18-18-07256.1998>