



## BDNF Pretreatment Attenuates Morphine-Induced Learning and Memory Impairment in Rats

Babaei Parvin (PhD)<sup>1,2\*</sup>, Vahdati Sanaz (MD Stu)<sup>2</sup>, Soltani-Tehrani Bahram (PhD)<sup>1</sup>

### ARTICLE INFO

**Article type:**  
Original Article

**Article history:**  
Received: 19 January 2014  
Accepted: 23 May 2014  
Available online: 20 March 2015  
CJNS 2015; 1 (1): 12-18

1. Cellular & Molecular Research Center,  
Faculty of Medicine, Guilan University  
of Medical Sciences, Rasht, Iran

2. Physiology Department, Faculty of  
Medicine, Guilan University of Medical  
Sciences, Rasht, Iran

**\*Corresponding author:**  
Physiology Department, Faculty of  
Medicine, Guilan University of  
Medical Sciences, Rasht, Iran  
Email: p\_babaei@gums.ac.ir

### ABSTRACT

**Background:** It has been known that Brain-Derived Neurotrophic Factor (BDNF) is involved in neural survival and long term memory (LTM). Here we hypothesized that BDNF as a potent neurotrophic factor might modulate amnesic effect induced by morphine.

**Objectives:** The aim of this study was to examine whether infusion of exogenous BDNF in the CA1 regions of the dorsal hippocampi could ameliorate memory impairment induced by morphine.

**Materials and Methods:** Forty rats were divided into 5 groups for dose response study of morphine (2.5, 5, 7.5 and 10 mg/kg morphine, and saline, intraperitoneal) on memory retention. For second part of the experiment 24 animals were divided into three groups: (morphine +BDNF, morphine + saline and saline + saline). Two weeks after stereotaxic surgery, animals received 0.5 µl bilateral infusion of either saline or BDNF (5 µg/rat) intrahippocampally, 30 minutes before morphine treatment (7.5 mg/kg, i.p.). Step-through inhibitory avoidance task has been used to examine retrieval of memory formation, 1.5 and 24 h after the training.

**Results:** The results showed that systemic administration of 7.5 and 10 mg/kg morphine compared with saline immediately after the training impairs long-term retention of memory for passive avoidance task in rats tested 24 hours later ( $p < 0.01$ ). Surprisingly intra-CA1 microinjection of BDNF 30 minutes prior to injection of morphine significantly prevented amnesia ( $p < 0.001$ ).

**Conclusions:** These findings suggested that increase the level of BDNF in the CA1 region of the hippocampus during 30 minutes time window before morphine administration might modulate morphine-induced amnesia.

**Keywords:** Brain-Derived Neurotrophic Factor; Amnesia; Morphine; Rats

Copyright © 2015 Caspian Journal of Neurological Sciences. All rights reserved.

➤ **Please cite this paper as:**

Babaei P, Vahdati S, Soltani-Tehrani B. BDNF Pretreatment Attenuates Morphine-Induced Learning and Memory Impairment in Rats. Caspian J Neurol Sci 2015; 1(1):12-18.

### Introduction

Morphine has been widely used in pain treatment, but its strong dependency potential is a serious challenge to its clinical usage. It is reported that opioid peptides, especially morphine

influence on the processes of learning and causes memory impairment. Impairment of memory has been reported after both chronic and acute morphine administration (1-4). Pre- or post-training administration of morphine

impairs specifically performance in the passive avoidance task (3, 5).

Memory is critically depended on the hippocampus region, and can be divided into short-term and long-term forms (6, 7). Consolidation of short term (STM) to long-term (LTM) memory takes place immediately following the training experience (8, 9). This critical period is influenced by different neurotransmitters including glutamate, acetylcholine and dopamine (10-13). Moreover, LTM strongly depends on protein synthesis cascades and neurotropic factors particularly BDNF (14-16). According to the previous reports hippocampal BDNF appears to be necessary for LTM formation in the different discrete periods, immediately after, 1.4 hour and 3.6 hours after training (17, 18). Although many studies proposed that BDNF is a key molecule mediating persistence and maintenance LTM, it is still unclear whether BDNF pretreatment is capable of ameliorating memory impairment. This study was conducted to answer to this question.

## Materials and Methods

### *Animals:*

Sixty four male wistar rats weighing 200-250g were used in this study. They had free access to food and water, and kept at  $24 \pm 2^\circ\text{C}$  under a 12h/12h light dark cycle. Each group consisted of 8 animals and each animal was tested once. All experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals (National Institute of Health Publication No.80-23, revised 1996) approved by the Research and Ethics Committee of Guilan University of Medical Sciences.

### *Surgery:*

The animals were anaesthetized via the intraperitoneal (i.p.) injection of ketamine and

xylazine (100 and 10 mg/kg, respectively), and fixed in the flat –skull position using stereotaxic apparatus (David Kopf Instruments, USA). The rats' scalp were cut, a small craniotomy was drilled and cannulas (22-gauge diameter) were bilaterally implanted into the CA1 region of the hippocampus at coordinates: AP – 3mm, L  $\pm$  2mm and V – 2.8mm (19).

### *Micro infusions:*

Morphine sulphate (Darupakhsh, Iran) and human recombinant BDNF (R&D, USA) were dissolved in sterile 0.9% saline. First, animals were divided into five groups (saline, morphine 2.5, 5, 7.5 and 10 mg/kg.) for dose response study of morphine. Secondly, 24 rats were divided into three experimental groups (morphine + BDNF, morphine + saline and saline + saline, n = 8 each) and underwent stereotaxic surgery. Two weeks later, animals received a 0.5  $\mu\text{l}$  bilateral infusion of saline or BDNF (2.5 $\mu\text{g}$ /0.5 $\mu\text{l}$ /side) intra - hippocampally 30 minutes before morphine treatment (7.5 mg/kg, i.p.).

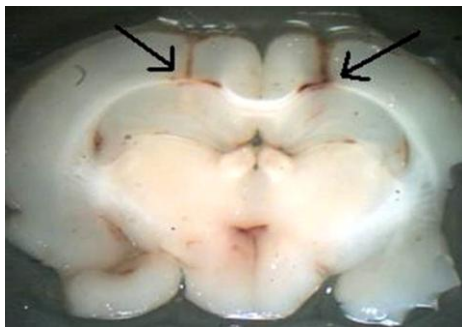
### *Inhibitory avoidance apparatus:*

The apparatus consisted of two equal size of compartments, one light and one dark (20 $\times$ 20 $\times$ 30 cm high), connecting via a guillotine door (7 $\times$ 9 cm). The floor of the dark compartment was made of stainless steel rods (2.5 mm in diameter) with a distance of 1 cm.

For the acquisition trial, rat was placed in the light compartment and the door between the two compartments was opened 20 seconds later. When the rat entered the dark compartment, the door closed and an electric foot shock (1 mA, 50 Hz, 5 seconds) was delivered through the grid floor. For the retention trial, the rat was again placed in the light compartment 1.5 and 24 hours following the acquisition trial. The latency time

(seconds) for entering the dark compartment was recorded.

Upon completion of the inhibitory avoidance test, each rat was deeply anesthetized and 1 ml of a 4% methylene-blue solution was bilaterally infused through the cannula into the CA1 (0.5ml/side). The animals were decapitated and the brains were removed and placed in formaldehyde for two days (10%). Then, the brains were sliced and the injection site was verified according to the Paxinos & Watson, brain atlas 2005(19), (Figure1).



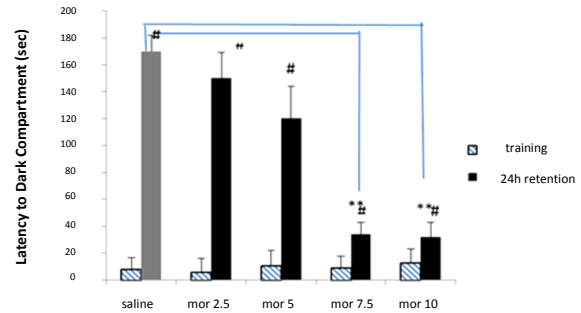
**Figure 1.** Photomicrograph from rat brain section showing the extension of the area reached by infusions into the hippocampus.

#### Data analysis:

Each value represents the mean  $\pm$  standard error of the mean (S.E.M.). After assaying the normality of data with Kolmogorov - Smirnov test, comparison of data among groups was performed using one-way analysis of variance with Tukey's post-test, when the *p-values* was  $< 0.05$ , the difference was considered to be significant. Calculations were performed using the SPSS statistical package version 19.

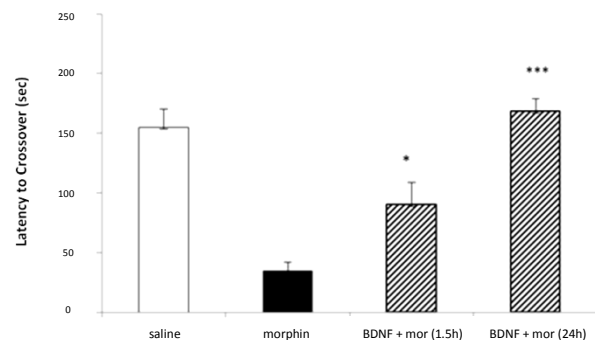
## Results

During the training trial, there was no significant difference among groups ( $p > 0.05$ , one way ANOVA). Systemic post-training administration of morphine (7.5 and 10 mg/kg) immediately after the training significantly decreased latency to enter to the dark compartment compared to the control receiving saline ( $p < 0.01$ ; Diagram 1).



**Diagram 1.** The effect of post-training administration of morphine on step-through latency. The rats ( $n = 8$  per group) received post-training saline (1 ml/kg, i.p.) or varying doses of morphine (mor) (2.5, 5, 7.5 and 10 mg/kg, i.p.) and were tested after 1.5 h and 24 h.  $**p < 0.01$  compared with the saline.

Following infusion of BDNF or saline, a 30 minutes wait-time and then an i.p. injection of morphine or saline, the rats performed the acquisition trial of the inhibitory avoidance test. Memory was assessed during the retention trial by measuring step-through latency in the passive avoidance task observed 1.5 and 24 hours after the acquisition trial (Diagram 2).



**Diagram 2.** The effect of acute BDNF pretreatment on memory consolidation prior to morphine injection. The latencies for the rat ( $n = 8$  per group) to enter to the dark room 1.5 h and 24 h after the training were expressed as mean  $\pm$  S.E.M.  $*p < 0.01$ ,  $***p < 0.001$  compared to morphine.

There was a strong significant group effect ( $F(2, 21) = 37$ ;  $p = 0.001$ , one way ANOVA) showing that the group receiving BDNF 30 minutes prior to morphine administration (BDNF + morphine) took longer to enter the dark chamber compared to either control group, saline + morphine or saline + saline.

## Discussion

In the current study, we showed that administration of morphine immediately after training impairs memory retention in the inhibitory avoidance test. Additionally, exogenous BDNF infusion into the hippocampal CA1 region, 30 minutes before morphine administration, ameliorated the morphine-induced memory impairment.

The impairment of memory induced by morphine is consistent with previous studies (3, 20, 21). Based on the immediate post-training administration of morphine and its short half-life in the brain (approximately 1 hour), it is likely that morphine is affecting the early phase of memory consolidation (22). Opioids produce their principal effects on memory by binding to at least three different types of receptors:  $\mu$ ,  $\delta$  and  $\kappa$  opioid receptors (3). All opioid receptor subtypes inhibit adenylyl cyclase and  $\text{Ca}^{2+}$  channels and stimulate  $\text{K}^+$  channels. These effects are required for morphine-induced amnesia in the passive avoidance test (23). Alternately, neuropharmacological studies have revealed that activation of opioid receptors may decrease the function of the cholinergic system (24-26). However, one cannot exclude the possibility that morphine interferes with other neurotransmitter systems, such as adrenergic or dopaminergic, to induce amnesia (27, 28).

Interestingly, we also showed that infusion of BDNF into the dorsal hippocampus 30 minutes before training was sufficient to prevent the amnesic effect of morphine as well as enhance memory consolidation compared to the saline control group. To our knowledge, this study was the first to demonstrate that BDNF attenuates morphine-induced memory impairment.

Animal and human studies suggest that hippocampal BDNF plays a major role in

memory by promoting neural plasticity (29, 30). Additionally, BDNF is required for the consolidation of short-term and long-term memory, especially in glutamatergic and GABAergic synapses (29, 31-33). Previous studies report an increase in BDNF mRNA in the dentate gyrus of the hippocampus following the acquisition trial (17, 34). Conversely, Alonso et al. (2002) showed that infusion of an anti-BDNF antibody impaired LTM, when given 15 minutes before or 1 and 4 hours after training (17). Our results were in agreement with the Johnston et al. study showing that injection of recombinant BDNF before training enhanced memory recall in day old chicks (35). Our findings confirmed that 30 minutes before the induction of amnesia was a sensitive time window which was critical for LTM formation, so it is more likely to relate this result to acute effects of BDNF on synaptic transmission, rather than protein synthesis due to inadequate time. Binding of BDNF to tropomyosin receptor kinase B (TrkB) triggers a number of intra-cellular signalling pathways from long lasting effects to enhancing early long term potentiation (LTP) and phosphorylation of synaptic proteins (36-39). Rapid  $\text{Ca}^{2+}$  influx through NMDA-type glutamate receptors and subsequent protein phosphorylation events modify pre-existing synapses and trigger early LTP, an important mechanism mediating memory formation (40, 41). A recent surprising study revealed suppressive effect of BDNF as a negative modulator on morphine reward (42). Some of the neurotransmitters regulate BDNF synthesis, and in turn they are regulated by BDNF. For example BDNF modulates acetylcholine, dopamine, and glutamate release in the hippocampus, depolarizes neurons and interferes in Ca signaling (43-51). The significance of these reciprocal regulations was intriguing and

could represent a novel framework into the molecular basis of morphine-induced amnesia.

For future studies we propose administration of antibody against BDNF and also different doses of BDNF prior to morphine injection.

## Conclusion

The present study shows that morphine impairs the consolidation phase of long-term recognition memory, possibly by preventing a learning-induced increase in BDNF levels in the hippocampus. This study suggests that intra-hippocampal infusion of BDNF prevents the deficit in memory consolidation caused by morphine.

## Conflict of Interest

No conflict of interest.

## References

1. Ma M, Chen Y, He J, Zeng T, Wang JH. Effects of Morphine and Its Withdrawal on Y-Maze Spatial Recognition Memory in Mice. *Neuroscience* 2007; 147(4):1059-65.
2. Castellano C, Pavone F, Allegra S. Morphine and Memory in DBA/2 Mice: Effects of Stress and of Prior Experience. *Behav Brain Res* 1984; 11(1):3-10.
3. Itoh S, Takashima A, Igano K, Inouye K. Memory Effect of Caerulein and Its Analogs in Active and Passive Avoidance Responses in the Rat. *Peptides* 1989; 10(4):843-8.
4. Izquierdo I, Bevilaqua LM, Rossato JI, da Silva WC, Bonini J, Medina JH, et al. The Molecular Cascades of Long-Term Potentiation Underlie Memory Consolidation of One-Trial Avoidance in the CA1 Region of the Dorsal Hippocampus, But Not in the Basolateral Amygdala or the Neocortex. *Neurotox Res* 2008; 14(2-3):273-94.
5. Khavandgar S, Homayoun H, Zarrindast MR. The Effect of L-NAME and L-arginine on Impairment of Memory Formation and State-Dependent Learning Induced by Morphine in Mice. *Psychopharmacology* 2003; 167(3):291-6.
6. Milner B, Squire LR, Kandel ER. Cognitive Neuroscience Review and the Study of Memory. *Neuron* 1998; 20:445-68.
7. Kumaran D, Maguire EA. The Human Hippocampus: Cognitive Maps or Relational Memory?. *J Neurosci* 2005; 25(31):7254-9.
8. Bekinschtein P, Cammarota M, Igaz LM, Bevilaqua LR, Izquierdo I, Medina JH. Persistence of Long-Term Memory Storage Requires a Late Protein Synthesis-and BDNF-dependent Phase in the Hippocampus. *Neuron* 2007; 53(2):261-77.
9. Castellano C, Cestari V, Ciamei A. NMDA Receptors and Learning and Memory Processes. *Curr Drug Targets* 2001; 2(3):273-83.
10. Camera K, Mello C, Ceretta A, Rubin MA. Systemic Administration of Polyaminergic Agents Modulate Fear Conditioning in Rats. *Psychopharmacology* 2007; 192(4):457-64.
11. Cammarota M, Bevilaqua LR, Rossato JI, Lima RH, Medina JH, Izquierdo I. Parallel Memory Processing by the CA1 Region of the Dorsal Hippocampus and the Basolateral Amygdala. *Proc Natl Acad Sci U S A* 2008; 105(30):10279-84.
12. Decker MW, McGaugh JL. The Role of Interactions between the Cholinergic System and Other Neuromodulatory Systems in Learning and Memory. *Synapse* 1991; 7(2):151-68.
13. Darvas M, Fadok JP, Palmiter RD. Requirement of Dopamine Signaling in the Amygdala and Striatum for Learning and Maintenance of a Conditioned Avoidance Response. *Learn Mem* 2011; 18(3):136-43.
14. Rossato J, Bevilaqua L, Izquierdo I, Medina JH, Cammarota M. Dopamine Controls Persistence of Long-Term Memory Storage. *Science* 2009; 325(5943):1017-20.
15. 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(6):e6007.
16. Schinder AF, Poo M-m. The Neurotrophin Hypothesis for Synaptic Plasticity. *Trends Neurosci* 2000; 23(12):639-44.
17. Alonso M, Vianna MR, Depino AM, Mello e Souza T, Pereira P, Szapiro G, et al. BDNF-triggered Events in the Rat Hippocampus Are Required for Both Short-and Long-Term

- Memory Formation. *Hippocampus* 2002; 12(4):551-60.
18. Igaz L, Vianna M, Medina J, Izquierdo I. Two Time Periods of Hippocampal mRNA Synthesis Are Required for Memory Consolidation of Fear-Motivated Learning. *J Neurosci* 2002; 22(15):6781-9.
  19. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. 5th ed. San Diego: Academic Press; 2004.
  20. Pavone F, Castellano C. Effects of Tifludom on Passive Avoidance Behaviour in DBA/2 mice. *Behav Brain Res* 1985;15(3):177-81.
  21. Zarrindast MR, Asadi F, Rezaeifard A. Repeated Pretreatment of Morphine Prevents Morphine-Induced Amnesia: A Possible Involvement for Dorsal Hippocampal NMDA Receptors. *Arch Iran Med* 2011; 14(1):32-8.
  22. Bouw M, Xie R, Tunblad K, Hammarlund-Udenaes M. Blood-Brain Barrier Transport and Brain Distribution of Morphine-6-Glucuronide in Relation to the Antinociceptive Effect in Rats—Pharmacokinetic/Pharmacodynamic Modelling. *Br J Pharmacol* 2001; 134(8):1796-804.
  23. Galeotti N, Ghelardini C, Bartolini A. Differential Prevention of Morphine Amnesia by Antisense Oligodeoxynucleotides Directed Against Various Gi-protein  $\alpha$  Subunits. *Br J Pharmacol* 2001; 133(2):267-74.
  24. Ukai M, Lin H. Involvement of  $\mu$  Opioid Receptors and Cholinergic Neurotransmission in the Endomorphins-Induced Impairment of Passive Avoidance Learning in Mice. *Behav Brain Res* 2002; 129(1):197-201.
  25. Li Z, Wu C, Pei G, Xu NJ. Reversal of Morphine-Induced Memory Impairment in Mice by Withdrawal in Morris Water Maze: Possible Involvement of Cholinergic System. *Pharmacol Biochem Behav* 2001; 68(3):507-13.
  26. Baratti C, Introini I, Huygens P. Possible Interaction between Central Cholinergic Muscarinic and Opioid Peptidergic Systems during Memory Consolidation in Mice. *Behav Neural Biol* 1984; 40(2):155-69.
  27. Homayoun H, Moghaddam B. NMDA Receptor Hypofunction Produces Opposite Effects on Prefrontal Cortex Interneurons and Pyramidal Neurons. *J Neurosci* 2007; 27(43):11496-500.
  28. Zarrindast MR, Farahmandfar M, Rostami P, Rezaeifard A. The Influence of Central Administration of Dopaminergic and Cholinergic Agents on Morphine-Induced Amnesia in Morphine-Sensitized Mice. *J Psychopharmacol* 2006; 20(1):59-66.
  29. Poo M. Neurotrophins as Synaptic Modulators. *Nat Rev Neurosci* 2001; 2(1):24-32.
  30. Lu Y, Christian K, Lu B. BDNF: A Key Regulator for Protein Synthesis-Dependent LTP and Long-Term Memory?. *Neurobiol Learn Mem* 2008; 89(3):312-23.
  31. Tyler WJ, Alonso M, Bramham C, Pozzo-Miller LD. From Acquisition to Consolidation: on the Role of Brain-Derived Neurotrophic Factor Signaling in Hippocampal-Dependent Learning. *Learn Mem* 2002; 9(5):224-37.
  32. Rutherford L, Nelson S, Turrigiano G. BDNF Has Opposite Effects on the Quantal Amplitude of Pyramidal Neuron and Interneuron Excitatory Synapses. *Neuron* 1998; 21(3):521-30.
  33. Vicario-Abejón C, Collin C, McKay R, Segal M. Neurotrophins Induce Formation of Functional Excitatory and Inhibitory Synapses between Cultured Hippocampal Neurons. *J Neurosci* 1998; 18(18):7256-71.
  34. Falkenberg T, Mohammed A, Henriksson B, Persson H, Winblad B, Lindefors N. Increased Expression of Brain-Derived Neurotrophic Factor mRNA in Rat Hippocampus Is Associated with Improved Spatial Memory and Enriched Environment. *Neurosci Lett* 1992; 138(1):153-6.
  35. Johnston A, Rose S. Memory Consolidation in Day-Old Chicks Requires BDNF but Not NGF or NT-3; an Antisense Study. *Brain Res Mol Brain Res* 2001; 88(1):26-36.
  36. Yoshii A, Paton M. Postsynaptic BDNF-TrkB Signaling in Synapse Maturation, Plasticity, and Disease. *Dev Neurobiol* 2010; 70(5):304-22.
  37. Alder J, Thakker-Varia S, Bangasser D, Kuroiwa M, Plummer MR, Shors TJ, et al. Brain-Derived Neurotrophic Factor-Induced Gene Expression Reveals Novel Actions of VGF in Hippocampal Synaptic Plasticity. *J Neurosci* 2003; 23(34):10800-8.
  38. Almeida R, Manadas B, Melo C, Gomes JR, Mendes CS, Grãos MM, et al. Neuroprotection by BDNF Against Glutamate-Induced Apoptotic Cell Death Is Mediated by

- ERK and PI3-kinase Pathways. *Cell Death Differ* 2005; 12(10):1329-43.
39. Jovanovic J, Thomas P, Kittler J, Smart TG, Moss SJ. Brain-Derived Neurotrophic Factor Modulates Fast Synaptic Inhibition by Regulating GABAA Receptor Phosphorylation, Activity, and Cell-Surface Stability. *J Neurosci* 2004; 24(2):522-30.
  40. Bliss T, Collingridge G. A Synaptic Model of Memory: Long-Term Potentiation in the Hippocampus. *Nature* 1993; 361(6407):31-9.
  41. Malenka R, Nicoll R. Long-Term Potentiation a Decade of Progress. *Science* 1999; 285(5435):1870-4.
  42. Koo JW, Mazei-Robison MS, Chaudhury D, Juarez B, LaPlant Q, Ferguson D, et al. BDNF Is a Negative Modulator of Morphine. *Science* 2012; 338 (6103):124-8.
  43. Knipper M, Penha BM, Blöchl A, Breer H, Thoenen H, Lindholm D. Positive Feedback Between Acetylcholine and the Neurotrophins Nerve Growth Factor and Brain-Derived Neurotrophic Factor in the Rat Hippocampus. *Eur J Neurosci* 1994;6(4):668-71.
  44. Blöchl A, Sirrenberg C. Neurotrophins Stimulate the Release of Dopamine from Rat Mesencephalic Neurons via Trk and p75Lnr Receptors. *J Biol Chem* 1996; 271(35):21100-7.
  45. Li Y, Zhang Y, Lester H, Schuman EM, Davidson N. Enhancement of Neurotransmitter Release Induced by Brain-Derived Neurotrophic Factor in Cultured Hippocampal Neurons. *J Neurosci* 1998; 18(24):10231-40.
  46. Jovanovic JN, Czernik AJ, Fienberg AA, Greengard P, Sihra TS. Synapsins As Mediators of BDNF-enhanced Neurotransmitter Release. *Nat Neurosci* 2000; 3(4):323-9.
  47. Carvalho AL, Caldeira MV, Santos SD, Duarte CB. Role of the Brain-Derived Neurotrophic Factor at Glutamatergic Synapses. *Br J Pharmacol* 2008; 153(S1):S310-24.
  48. Kang H, Schuman EM. Long-Lasting Neurotrophin-Induced Enhancement of Synaptic Transmission in the Adult Hippocampus. *Science* 1995; 267(5204):1658-62.
  49. Vaynman S, Ying Z, Yin D, Gomez-Pinilla F. Exercise Differentially Regulates Synaptic Proteins Associated to the Function of BDNF. *Brain Res* 2006;1070(1):124-30
  50. Blum R, Kafitz KW, Konnerth A. Neurotrophin-Evoked Depolarization Requires the Sodium Channel NaV1. 9. *Nature* 2002; 419(6908):687-93.
  51. Rose CR, Blum R, Pichler B, Lepier A, Kafitz KW, Konnerth A. Truncated TrkB-T1 Mediates Neurotrophin-Evoked Calcium Signalling in Glia Cells. *Nature* 2003; 426(6962):74-78.