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Original Article

Changes in the levels of hippocampal BDNF expression are accompanied with inflammatory dental pain-induced learning and memory impairment

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Abstract

Introduction: Learning and memory requires a brain-derived neurotrophic factor (BDNF)-dependent phase in the hippocampus. It has been reported that chronic pain decreases hippocampal BDNF levels. We have also previously reported that noxious stimulation of the rat tooth pulp impairs learning and memory. Therefore, we decided to find the changes in the hippocampal BDNF expression which are associated with tooth pain and learning and memory impairment.

Methods: Dental pulp nociception was induced by intradental injection of capsaicin (100µg) in male Wistar rats. BDNF expression levels were determined by semi-quantitative RT-PCR and western blotting.

Results: The data indicated that capsaicin elicited pain behaviors and impaired learning and memory in Morris water maze test. The protein and mRNA levels of BDNF were significantly (P<0.05) decreased in capsaicin-treated rats as compared with control animals. Furthermore, iboprofen (120mg/kg, ip) treatment caused a significant (P<0.05) up-regulation of the BDNF protein and mRNA in the hippocampus of capsaicin-injected animals.

Conclusion: These findings suggest that inflammatory dental pain induces hippocampal function impairments by decreasing in BDNF expression.

Keywords:
Dental pain;
Learning and memory;
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Introduction

Cognitive functions are affected by pain in both human and animals (Moriarty and Finn, 2014). Pain-induced learning and memory deficits have been reported in several studies (Apkarian et al., 2004; Dick and Rashiq, 2007; Kooshki et al., 2016). We have previously reported that pulpal pain can impair spatial learning and memory in male rats in the Morris water maze (MWM) test (Raoof et al., 2015; Amirkhosravi et al., 2015). Although the precise mechanisms of pain-related cognitive impairment have not yet been elucidated, a number of possible mechanisms for this phenomenon may include synaptic plasticity, down-regulation of neurotrophic

factors and their receptors (Kozlovskiy et al., 2012), elevated pro-inflammatory cytokines (Khairova et al., 2009) and alterations in cannabinoid receptor function (Chevaleyre et al., 2006).

The hippocampus is an essential site for learning and spatial memory processing, and it is one of the few brain regions to display adult neurogenesis. It has been hypothesized that adult hippocampal neurogenesis is also involved in hippocampal-related learning and memory (Leuner et al., 2006). Damage to the hippocampal structures has been also associated with learning and memory impairments (Squire, 1992). It is likely that hippocampal vulnerability may result from cellular components involved in hippocampal apoptosis and neurogenesis (Kuhajda et al., 2002). We have previously reported that apoptotic factors are over-expressed in the hippocampus of adult male rats suffering from inflammatory pulpal pain (Raoof et al., 2015). However, the exact cellular mechanisms underlying the vulnerability of hippocampus remain to be elucidated.

The involvement of neurotrophic growth factors in the underlying mechanism of hippocampal neurogenesis has been much reported recently (Lee and Son, 2009; Leal and Yassa, 2015). Adult hippocampal neurogenesis is positively affected by neurotrophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin-3. Among these, BDNF has been intensively shown to be involved in learning, memory and synaptic plasticity (Poo, 2001; Leal and Yassa, 2015). Surprisingly, spatial learning and memory is impaired in a BNDF-deficient animal model (Mu et al., 1999; Petzold et al., 2015).

Since the role of BDNF on the decreased hippocampal function following orofacial pain has not been fully elucidated, the present study was designed to analyze the expression level of BDNF in the hippocampus of dental pain suffering and dental pain-induced cognitive impaired rats.

Materials and methods

Animals

Experiments were carried out on adult male Wistar rats weighing 250-300g. The animals were kept under controlled temperature (24±1°C) and 12-h light-dark cycle, four per cage and had free access to food (standard rodent diet) and water. All experimental protocols and treatments were approved by the Ethics Committee of Kerman Neuroscience Research Center (Ethics Code: EC/94). All efforts were made to minimize the number and suffering of animals in all steps of the study.

Nociceptive behavior

Pain behaviors were monitored between 10:00 and 13:00 am in a quiet room maintained at 23–24°C. Before capsaicin injection, each animal was placed in the test box for a 30-min habituation period to minimize additional stress. The rats did not have access to food or water during the test.

Immediately following the injection, each rat was placed back in the transparent Plexiglass box (25×35×35) with a transparent floor positioned over a mirror at an angle of 45 degrees to allow for observation of nociceptive behavior. The rats' behavior was observed for 21 minutes, divided into 7 blocks of 3 minutes. Pain scores were determined for each block by scoring the animal behaviors presented each of the following responses which represents the same scoring criteria as Chidiac et al. (2002) study: 0– Calm, normal behavior such as grooming; 1– Abnormal head movements such as mild head shaking or continuous placement of the jaw on the floor or the wall of the cage; 2– Abnormal continuous shaking of the lower jaw; 3– Excessive rubbing of the mouth with foreleg movements, such as head grooming, but concentrated consistently and mainly on the lower jaw. A video camera was used to record the behavioral response.

Morris water maze test

Spatial learning was evaluated using a modified version of MWM. Briefly, the experimental apparatus consisted of around water tank (140cm wide and 45cm high) filled with water (25°C) and surrounded by visual cues around the tank. In the MWM task, a platform (diameter, 20cm; height, 32cm; and depth, 1-2cm below the surface of the water) was located in the center of one of the four quadrants. Data were collected automatically by a video-image motion analyzer (Ethovision, *Version* 3.1, Noldus Information Technology, Netherlands). The animals were exposed to the swimming pool without the platform for 2min one day before, aiming to habituate them to the experimental procedure and reduce their stress.

Tissue extraction and preparation

The rats were anesthetized (exposed to a CO_2 atmosphere) and decapitated and the brains were removed immediately. Brains were dissected along the sagittal midline, followed by bilateral removal of the hippocampus. The hippocampus was immediately placed on ice in a glass petri dish. Tissue samples were weighed and immediately frozen in liquid nitrogen and stored at −70°C until assay. The dissected hippocampus from each rat was randomly distributed for further western blot and RT-PCR assays.

mRNA analysis

Total cellular RNAs were isolated from the hippocampus by a modification of the guanidine isothiocyanate–phenol–chloroform method using RNX+ reagent. A semiquantitative RT-PCR reaction was performed using Oligo-dT primer and M-MuLV reverse transcriptase. Three separate PCR reactions were used for studying gene expression in the samples obtained from each rat. Each PCR reaction was carried out using selective forward and reverse primers for β-actin (as an internal standard) and BDNF proteins.

The sequence of the primers used was: BDNF forward: 5′-GAC GAC GAC GTC CCT GGC TGA-3′, BDNF reverse: 5′-ACG ACT GGG TAG TTC GGC ACT GG-3′; β-actin forward: 5′-CCC AGAGCA AGA GAG GCA TC-3′, β-actin reverse: 5′-CTC AGG AGG AGC AAT GAT CT-3′.

Taq DNA polymerase (Cinaclon, Iran) used for DNA amplification and reactions were set up according to the manufacturer's protocol. The PCR reactions were incubated at 94°C for 5 min, followed by 25 cycles of thermal cycling (45s at 94°C, 45s at 55°C and 45s at 72°C). The final cycle was followed by a 5min extension step at 72°C. The reaction parameters were adjusted to obtain a condition with a linear relation between the number of PCR cycles and PCR products and with linear relation between the initial amount of cDNA template and PCR product. PCR products bands were quantified by densitometry using LabWorks analyzing software (UVP, UK).

Western blot analysis

Rat hippocampal tissues were lysed in RIPA buffer containing 10 mM Tris–HCl, pH 7.4, 150mM NaCl, 1mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.1% Na-deoxycholate, 1% NP-401% NP-40 and protease inhibitors (1mM phenylmethylsulfonyl fluoride, 2.5μg/ml of leupeptin, 10μg/ml of aprotinin) and 1mM sodium orthovanadate. Equal amounts of protein from each sample (40μg) were separated using SDS–PAGE gel and transferred to PVDF membrane. The blots were then blocked with 3% non-fat milk in 0.1% Tween-Tris-buffered saline for 2h at room temperature, followed by overnight (4°C) incubation with BDNF primary antibody (1:15,000, Santa Cruz biotechnology, USA). The primary antibody was detected with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:15,000, Santa Cruz biotechnology, USA). Blots were developed with the chemiluminescence reagent its detection film. Quantification of western blot signals was conducted by densitometry analysis using images J software.

Statistical analysis

Data are presented as mean±standard error of mean (SEM). Differences in pain scores, learning and memory indices and the amount of BDNF mRNA and protein levels between experimental groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's test. $P<0.05$ was considered significant.

Results

Assessment of pain behaviors in experimental groups

As a model of inflammatory orofacial pain, we used 100μg injections of capsaicin into the dental pulp, which produced nociceptive behaviors (Fig. 1). Ibuprofen pretreatment (120mg/kg, intragastrically) significantly decreased capsaicin-induced pain scores.

Assessment of spatial learning and memory in experimental groups

Inflammatory pulpal pain significantly ($P<0.001$) increased time to find the platform as compared to intact and sham-vehicle animals in hidden platform trials. Ibuprofen completely inhibited the effect of capsaicin in this test (Fig. 2A). As shown in Figure 2B, traveled distance was also significantly increased in capsaicin-treated group which was reversed by

ibuprofen treatment.

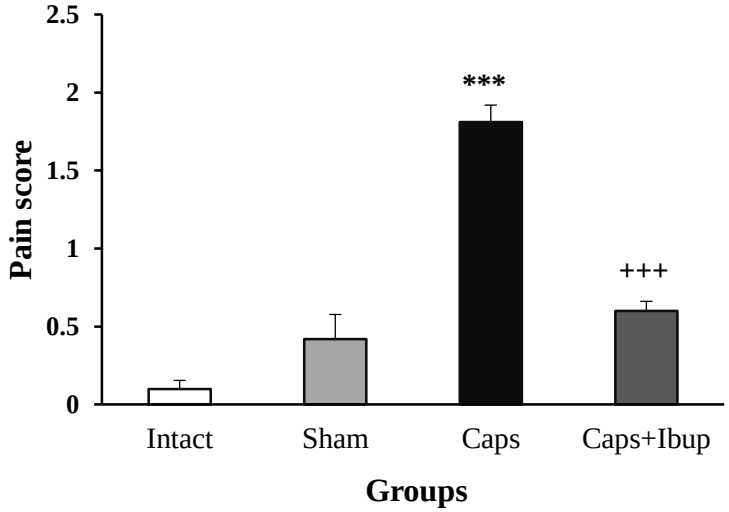

Fig.1. Dental pain score in control (intact), sham, capsaicin and capsaicin plus ibuprofen-treated groups. Each value in the graph represents the mean±SEM. $^{***}P<0.001$ versus intact and sham groups. $^{+++}P<0.001$ versus capsaicin-treated (Caps) animals.

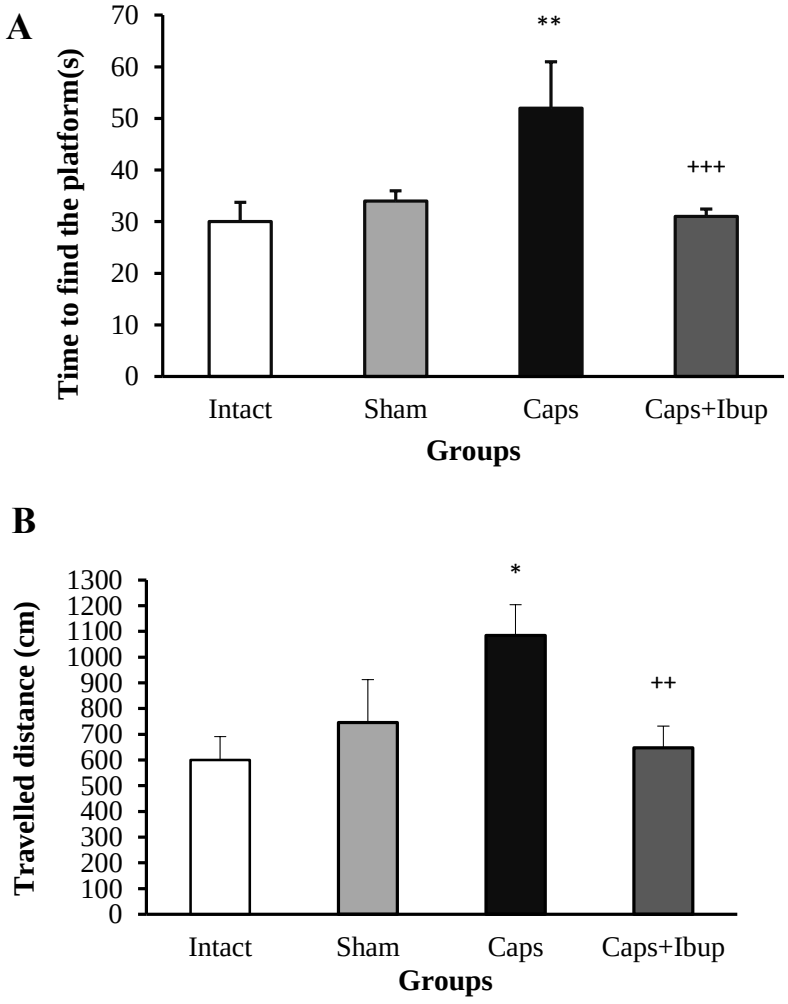

Fig.2. Comparison of time (A) and travelled distance (B) to find the Morris water maze platform between study groups. One-way analysis of variance was used to compare the multiple group means followed by Tukey's test. Each value in the graph represents mean±SEM. $^{*}P<0.05$ and $^{**}P< 0.01$ versus intact and sham groups. $^{++}P<0.01$ and $^{+++}P<0.001$ versus capsaicin-treated (Caps) animals.

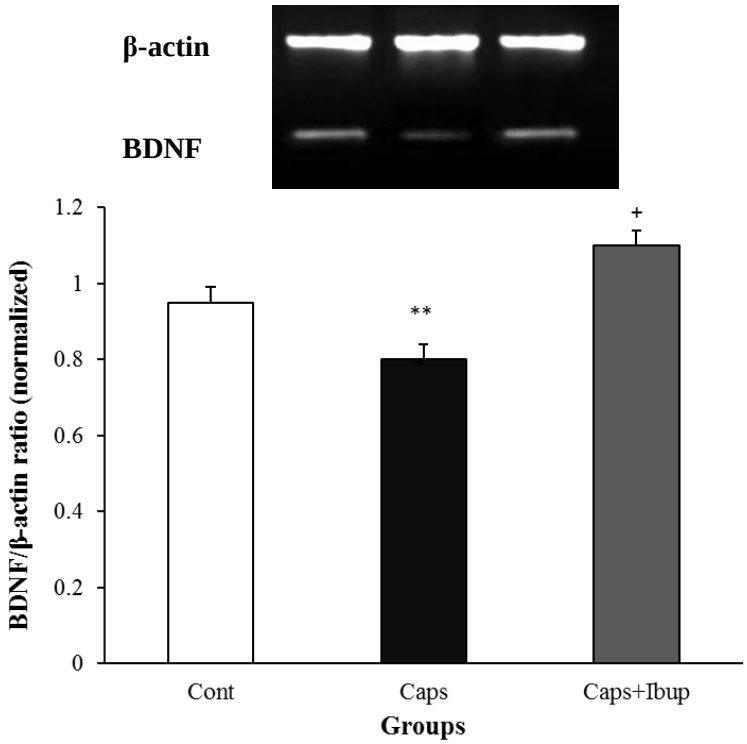

Fig.3. Comparison of BDNF gene expression (mRNA) level between study groups. One-way analysis of variance used. Values represent mean±SEM. $^{**}P<0.01$ versus control animals. $^{\#}P<0.05$ versus capsaicin-treated group.

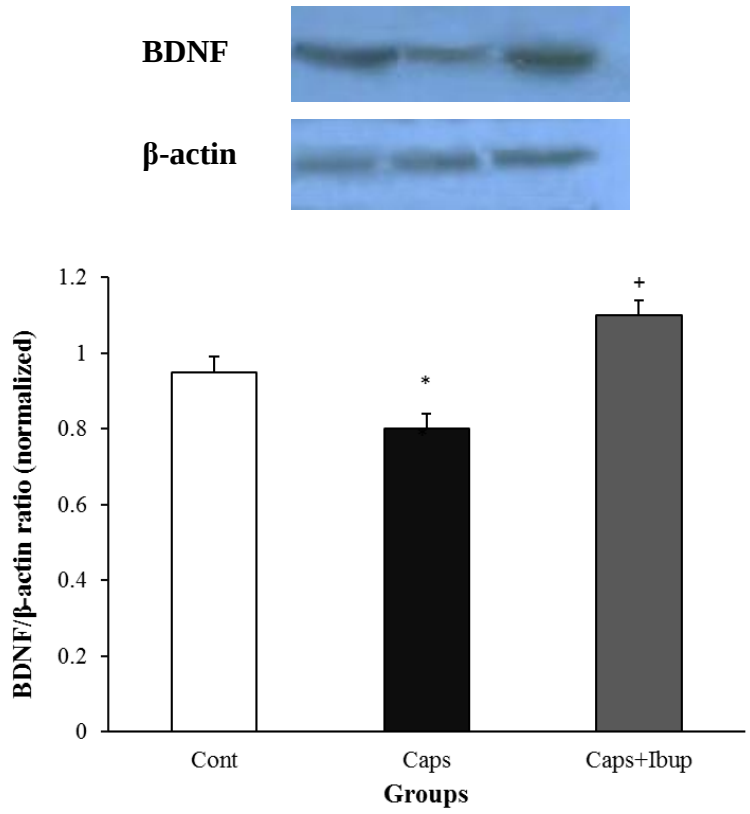

Fig.4. Comparison of BDNF protein level between study groups. One-way analysis of variance used. Values represent mean±SEM. $^{*}P<0.05$ versus control group. $^{+}P<0.05$ versus capsaicin-treated group.

Dental pain-evoked down-regulation of BDNF expression in the hippocampus

Twenty-four hours after the intradental injection of capsacin, hippocampal BDNF mRNA levels were significantly decreased by 33% (Fig. 3). BDNF expression in ibuprofen-treated rats was similar to sham controls group. Figure 5 shows the nociceptive regulation of the BDNF protein; however, BDNF

protein was down-regulated in pain suffering animals and restored to the basal control levels after ibuprofen treatment.

Effect of tooth inflammatory pulpal pain on BDNF protein level in hippocampus

Inflammatory tooth pain induced by intradental application of capsaicin (100μg/rat) could decrease the level of BDNF in hippocampus (P<0.05); however, in ibuprofen-pretreated rats, BDNF protein level was closed to that in control groups (P>0.05, Fig 4).

Discussion

To test the potential distractive effect of orofacial pain on learning and memory, we used male rats which had inflammatory dental pain. Molecular assays showed that dental pain could decrease the hippocampal levels of BDNF mRNA and protein. Meanwhile, administration of ibuprofen attenuated hippocampal BDNF decrement. It has been demonstrated that BDNF is crucial for the maintenance of neural plasticity during aging and in neurodegenerative disease (Xu et al., 2000). Therefore, hippocampal BDNF can mediate the effect of pain on cognitive functions.

The role of BDNF on hippocampus-dependent learning and memory has been reported in numerous conflicting reports. It seems the hippocampus, which is required for many forms of long-term memory in humans and animals, appears to be an important site of BDNF action. It has been documented that BDNF has an improving role in hippocampal-dependent learning and memory (Pisu et al., 2011; Lubin, 2011). In contrast, some investigations showed that central administration of BDNF has no effect on the learning rate of the spatial learning-impaired rats (Linnarsson et al., 1997). Formalin induced pain as well as complete Freund's adjuvant induced inflammation in rats caused significant reduction on neurogenesis in the hippocampal dentate gyrus and levels of both NK-1 receptor and BDNF mRNAs (Duric and McCarson, 2006).

Rapid and selective induction of BDNF expression has been observed in the hippocampus during contextual learning (Binder and Scharfman, 2004). Chronic pain stress in neonatal rats caused impairment in the spatial learning and memory in MWM test. The Bcl-2 and BDNF mRNA expressions decreases in the hippocampus of pain-suffered rats (Li et al., 2005).

It has been reported that the exposure to chronic restraint stress increases the adrenal gland weight and decreases the hippocampal BDNF levels (Macedo et al., 2015). After neuropathic pain induction by a chronic constriction injury of the sciatic nerve, hippocampal CA1 region BDNF levels is decreased (Saffarpour et al., 2017). However, dental pain-induced stress may be involved in the changes of BDNF in this study.

BDNF has a modulatory action at hippocampal synapses and at the first pain synapse between primary sensory neurons and spinal dorsal horn neurons. Actually hippocampal and sensory neurons share some properties for the release of endogenous BDNF. The binding of BDNF to the high-affinity TrkB receptors is essential for the induction of long-term potentiation as well as synaptic plasticity in the hippocampus (Malcangio and Lessmann, 2003). Rats hind paw inflammatory injection caused significant increase in BDNF mRNA levels in the ipsilateral dorsal horn, surprisingly, an opposite effect was observed in the hippocampus (Duric and McCarson, 2007).

It has been indicated that there are same plasticity mechanisms both in pain and learning and memory processes. BDNF is required for the hippocampus long-term potentiation (LTP) induction, and also hippocampal dendritic BDNF expression have a crucial for maintenance of late phase LTP (Lu et al., 2008). Acute application of exogenous BDNF increases evoked responses at the sites of BDNF and TrkB expression. Granule cell axons (mossy fibers) of dentate gyrus show the strongest BDNF immunoreactivity in brain (Rudge et al., 1998). Study of the transgenic mice over-expressing BDNF shows that normal function of the brain need to limited range of BDNF level (Croll et al., 1999).

It has been shown that pain elicits substantial changes in the hippocampal dendritic structure including morphology, length and arborization and spine density. In addition, levels of BDNF and the presynaptic proteins are indicators of abnormal neural plasticity (Tajerian et al., 2014). The release of neurotrophins from neurons is constitutive as well as activity dependent. High-frequency synaptic discharge is necessary for inducing BDNF release

from both presynaptic and postsynaptic elements of hippocampal neurons. More recent evidence indicates that BDNF may act postsynaptically in dentate granule cells of hippocampus (Malcangio and Lessmann, 2003).

Furthermore, exogenous BDNF facilitates the release of glutamate and NMDA-receptor-mediated synaptic transmission in the hippocampus (Riedel et al., 2003). Therefore, it's logic that BDNF down-expression can be attributed in the induction of learning and memory deficit by pain.

Application of nonsteroidal anti-inflammatory drug and a tricyclic antidepressant drug on pain- and stress-evoked gene expression in the rat spinal cord dorsal horn and hippocampus showed blocking of both pain- and stress-evoked alterations in hippocampal and spinal NK-1 and BDNF gene expression (Duric and McCarson, 2006). It has been demonstrated that pain-related plasticity has a crucial role for the recovery and survival of the organisms from the injury (Price and Inyang, 2015).

Conclusion

Learning and memory deficits as well as changes in neurotrophic factors have been previously reported in association of different type of pain. Taken together, the data demonstrate that changes in the hippocampal BDNF expression are accompanied with dental tooth inflammatory pain and may play a crucial role in pain-induced memory dysfunction; however further studies are needed to clarify the detailed mechanism.

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Conflict of interest

There is no conflict of interest.

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