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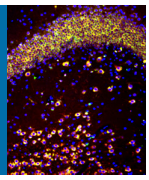

Original article

The function of BDNF in rats subjected to conditioning leison in the spinal cord or cortex associated with ERK1 pathway

Piao Zhang[1#], Chao-Hui Liang[1#], Di-ke Qiu[1], Yuan Jin[1], Cheng-Fei Bi[1], Yu-Shi[1], Liu-Lin Xiong[2], Jia Liu[1*]

1. Institute of Neuroscience, Kunming Medical University, Kunming 650000, China.
2. Translational Neuroscience Center, West China Hospital, Sichuan University, Chengdu 610041, PR China.
#They contribute equally to this work.

Abstract

Backgroud: Spinal cord and cortex with conditioning leison are a kind of neurological trauma which seriously jeopardized the health of human beings. They are devastating for society and individuals, in part owing to the high rates of disability and resulting medical costs. BDNF is a vital molecular, which play a critical roel in the progress of spinal cord or cortex repairment. Interestingly, we found ERK1 pathway was involving in the regulating process. But regretfully, the possible mechanism is ambiguous. **Methods:** Hence, in order to illuminate the theory, we established the model with sciatic nerve and spinal cord transection. And then spinal cords and cortexes were harvested from rats with sciatic nerve and spinal cord transection for performing RT-PCR and Western Blot, which aimed to detect the level of BDNF and ERK1. **Resluts:** The results manifested that the expression of BDNF and ERK1 was higher in sciatic nerve transection and spinal cord transection group (RSNTASCT/LD) than sciatic nerve transection one. **Conclusion:** Here, we may draw a conclusion that spinal cord will restrain the level of BDNF, and sciatic nerve transection can promote ERK1 expression. Moreover, sciatic nerve transection and spinal cord transection will promote ERK1 expression. Nevertheless, sciatic nerve transection and spinal cord transection will promote BDNF and ERK1 expression, whilst sciatic nerve transection can restrain ERK1 expression in cortex. In our data, we are the first time to expound the role of endogenous BDNF relating to the regeneration of spinal cord in the condition of peripheral nerve injury, and reveal the possible underlying mechanism of ERK1 pathway, which may provide certain theoretical basis and experimental basis for the treatment of spinal cord injury and prognosis in clinic.

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Corresponding author:
Dr. Jia Liu
Email: liujia aixuexi@163.com

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Introduction

In recent years, with the increasing of traffic accidents and sport injury, spinal cord injury (SCI) has become a common disease. The incidence rate of SCI in Britain and America were 12 people per million population and 30-32 people per million population, 7600~10000 cases increased in America in every year, while only in Taipei, Taiwan province in our country, the incidence of SCI was 14.6 people per million population (Coggrave M, et al., 2009; Feng HY, et al., 2011; Yeh YS, et al., 1993) . The occurrence of SCI has brought burden of economy and spirit for family and society. After SCI, patients often show motor neuron damage under the level of injury that included the flaccid paralysis with characteristics of decline of muscular tension, amyotrophia and negative pathological reflex (Rakowicz M, et al., 1989) (Piliavskiĭ AI, et al., 1988). They also show motor neuron damage above the level of injury that included the spastic paralysis characteristics of increasing of muscular tension, deep hyperreflexia and positive pathological reflex. SCI, a kind of severe trauma of central nervous system, whose main clinical manifestations are the complete loss of sensory and motor function below the level of injury, are divided into primary and secondary damage (Dreval' ON, et al., 2007). Conditioning lesion is a kind of injury that central nervous system is damaged on the premise of peripheral nerve injury (Rossignol S, et al., 2004). Some previous study found that conditioning lesion can promote the regeneration of neurons in damaged area and thought that the enhancement of regeneration of neurons induced by this lesion was associated with regeneration of neurons in damaged area , but the specific mechanism is unclear (Neumann S, et al., 2005; Neumann S, et al., 1999).

Brain-derived neurotrophic factor (BDNF), was found in the brain of pig in 1982 by Barde, a nerve chemist (BDNF) (Anita E, et al., 2012). In addition, BDNF, alkaline protein molecule, is a member of the family of neurotrophic factors with the molecular weight of 13.15KD and mainly expressed in brain (Yan Q, et al., 1997). The mRNA

expression level of BDNF was gradually declined from embryonic stage to maturity in spinal cord. The BDNF protein was mainly expressed in anterior horn motor neurons in maturity stage which suggest that BDNF may be related to the development and maintain of spinal cord (Maisonpierre PC, et al., 1990; Dougherty KD, et al., 2000; Zhou XF, et al., 1994; Goto A, et al., 1995) Dougherty found that BDNF was increased in local damage after SCI, the positive numbers of macrophages and astrocytes were obviously increased (Dougherty KD, et al., 2000).In the spinal cord transection, BDNF positive expression was also increased. These studies suggested that BDNF involved in repair of spinal cord and regeneration of neuron axon after SCI. BDNF was expressed in astrocyte after SCI suggesting that it may be related to neurotrophic factor produced by astrocyte that involved in the regeneration of neurons after SCI. The study found that BDNF mainly had nerve protective effect on neurons in injury area in the early SCI, while BDNF had nerve repair effect on injury area of spinal cord in the later part of SCI. Moreover, neuronal somas in the head of injury were obviously increased than that in the bottom of injury (Uchida K, et al., 1998; Ikeda O, et al., 2001).In addition, Sharma found that BDNF relieved edema by downregulating the expression of heat shock protein (HSP) and Heme Oxygenase (HO-2) after SCI (Sharma SH, et al., 2000).

Nowadays, exogenous BDNF has become a hotspot in the field of neuroscience research, and some of its research results have been already used for the treatment of SCI in clinic, such as the combination of BDNF and cell transplantation, intrathecal local injection of exogenous BDNF and so on. However, the expression and change of endogenous BDNF had more important effect on the repair and regeneration of neurons after SCI than that of exogenous BDNF, but the specific mechanism remains unclear (He BL, et al., 2013; Wang YF, et al., 2008).

Extracellular signal-regulated kinase (ERK) is a member of mitogen-activated protein kinase (MAPK). MAPK, a kind of intracellular serine/threonine protein kinase, can conduct various signals from extracellular to intracellular to exert its biological effect(Barrio-Real L, et al., 2014). The MAPK pathway can be divided into MAPK kinase (MAPKK/MKK), MAPK kinase-kinase (MAPKKK/MKKK) and MAPK according to the order of activation. They have common structural features, such as there are tripeptide motif in homologous VIII subregion in their catalytic area, their biggest activation all requires threonine and tyrosine in tripeptide motif to be phosphorylated and then activated by serine/threonine in the upstream (Crews CM, et al., 1992) (Zheng CF, et al., 1993). MAPK family was divided into four members, ERK including ERK1 and ERK2, p38 MAPK, c-Jun N-terminal kinase (JNK) and ERK5/BMK1 (Adams JP, et al., 2002). In addition, ERK was firstly found in the MAPK family. With the deepening of the research, researchers found that ERK can be activated by various ways, such as physiology and pathology, so as to exert its cell activation, proliferation, apoptosis resistance and so on. Previous study showed that BDNF protected the neonatal brain from hypoxic-ischemic injury in vivo via the ERK pathway (Han BH, et al., 2000).Moreover, the transplantation of olfactory ensheathing cells (OECs) resulted in neuropathic pain associated with BDNF regulated ERK activity in rats following cord hemisection (Lang BC, et al., 2013). But whether BDNF have effect on conditioning lesion via ERK pathway remains obscure.

Therefore, in order to observe the effect of this injury to the survival of neuronal soma and the regeneration of axon in injured area conditioning lesion model was built. In detail, spinal cord transection was performed seven days after sciatic nerve transection . Moreover, the function and effect of BDNF in this injury and the potential mechanism of BDNF and its downstream signaling molecule ERK were explored. Thus this experiment can provide new thought and theoretical basis for further studying on the regeneration of neurons after SCI and the treatment of SCI in clinic.

Materials and Methods

Animals and Grouping

Eighteen healthy female SD rats, weighing 250±20g, were purchased from animal experiment center of Kunming medical college and randomly divided into sham group, simple right sciatic nerve transection group, right sciatic nerve transection and spinal cord transection group, six rats within each group. Guidelines of caring laboratory animals and safety from NIH were followed. All animals were raised in plastic cages (n=2/cage) with soft bedding and free access to food and water in a temperature (21-25ºC) and humidity (45-50 %)-controlled room.

Animal Models

Simple Right Sciatic Nerve Transection

After SD rats were anesthetized by intraperitoneal injection of 3.6% chloral hydrate, they were fixed by prone position. Then, their skins were routinely prepared in the area of operation, and disinfected using iodine. The skins were cut in line along the inner thigh of rats and subcutaneous fascia was separated to expose the right sciatic nerve. Sciatic nerve was bluntly separated using a glass rod, surgical suture was threaded from the proximal sciatic nerve to the distal sciatic nerve, respectively. The separated sciatic nerve was cut from the middle of it using ophthalmic scissor, followed by the ends of sciatic nerve were ligated using surgical suture. Next, after little powdery Penicillium-Citrinum was added into partial operational place to prevent infection, muscles and skins were sutured step by step. Finally, rats were housed in the clean cages with free access to food and water and kept in warm condition. Postoperative rats were alone cultured in common cages, given anti-infection, and carefully nursed.

Right Sciatic Nerve Transection and Spinal Cord Transection

Right sciatic nerve was transected as previously described. Seven days later, these rats were anesthetized by intraperitoneal injection using 3.6% chloral hydrate again to perform spinal cord transection. After these rats were fixed by prone position, their skins in operational area were prepared, and disinfected using iodine. After position was determined on the skin of back through vertebral spines, about 3cm incision was cut in order to expose T9-11

vertebral spines. Then, T9-T11 vertebral spines were bitted. The border of T10 and T11 was completely transected using microscopic shear. Then, after little powdery Penicillium-Citrinum was added into partial operational place to prevent infection, muscles and skins were sutured step by step. Finally, rats were housed in the clean cages with free access to food and water and kept in warmcondition. Postoperative rats were alone cultured in common cages, given anti-infection, and carefully nursed. The sham rats received exposion of sciatic nerves and spinal cords without transection.

Tissues Harvest

7 days post-operation, tissues in the sham group and simple right sciatic nerve transection group were obtained. In sciatic nerve transection and spinal cord transection group, tissues were obtained at 7 days after sciatic nerve transection and 3 days after spinal cord transection.

RT-PCR

After total RNAs were extracted from spinal cords and cortexes of rats with different treatments using Trizol, they were reverse transcribed into cDNA. Then, BDNF and ERK were amplified through different mixtures that included 12.5 μL 2×PCR Master Mix, 0.5 μL upstream primer, 0.5 μL downstream primer, 10.5 μL PCR water nucle-free and 1 μL cDNA temple. And the primers used in this experiment were described in Table 1. Then the mixtures were reacted at 94ºC for 5 min, cycled 35 times at 94 ºC for 1 min, 55ºC for 1 min, 72ºC for 1 min, and finally at 72ºC for 10 min. At last, PCR products were run in 1% agarose gel electrophoresis and took pictures.

Western Blotting

Spinal cord tissues (250g) were added 1ml precooling protein extraction reagent which contained 98% RIPA lysis buffer (Beyotime, Jiangsu, China) and 2% cocktail pill (Roche), and homogenized on ice using in situ homogenate machine so that tissue mass were invisible. After the mixture was ice-bathed for 30min and blended every 10 minutes, the mixture was quashed for 5 s every 5 s, total 10 times in Ultrasonic Cell Breaking Machine. The lysate was centrifuged at 12000g for 15 min at 4ºC; then, the supernatant was collected, and the concentration of protein was curtained by BCA protein assay lit (Beyotime Institute). After that the precipitated proteins (80 μg) were separated on a SDS-PAGE gel at 350 mA for 2h and transferred to PVDF membranes at 350 mA for 2 h. SDS-PAGE was consisted of 10 mL 15% separation gel and 6 ml 5% spacer gel. 10 mL 15% separation gel consisted of 2.3 ml ddH2O, 5 ml 30% polyacrylamide, 2.5ml 1.5mol/L Tris (PH8.8), 0.1ml 10% sodium dodecyl sulfate (SDS), 0.1 ml 10% ammonium persulfate (AP) and 0.004 ml TEMED. 6 mL 5% spacer gel were consisted of 4.1 ddH2O, 1.0 ml 30% polyacrylamide, 0.75ml 1.5mol/L Tris (PH6.8), 0.06ml 10% SDS, 0.06 ml 10% AP and 0.006ml TEMED. After the transfer was finished, the PVDF membranes were washed in 1×TBS, then placed in 5% nonfat milk seen as sealed liquid and slowly swayed for 2 h on horizontal pendulum table. Then the PVDF membranes were incubated with primary antibodies of BDNF (Goat, Santa Cruz, 1:500) ERK1 (Rabbit, Abcam, 1:500) and β-actin (mice, Cell signaling, 1:2000) overnight at 4ºC, respectively. After the PVDF membranes were rapidly washed three times in 1×TBST for five minutes on horizontal pendulum table, the PVDF membranes were incubated with the secondary antibody Abexcel (anti-rabbit, Abcam, 1:1000) for 2 h with bobble at room temperature on horizontal pendulum table. Afterwards, the PVDF membranes were washed three times in 1×TBST for five minutes on horizontal pendulum table, and developed in Alpha Innotech (BioRad) with ECL. The processes of western blotting for cortexes are similar with that for spinal cords.

Statistical Analysis

Data were expressed as mean ± standard deviation. Experimental data were compared by one-way analysis of variance and repeated analysis of variance using SPSS17.0 software. $P< 0.05$ was considered as statistical difference.

Results

The Comparison of BDNF mRNA in Spinal Cord of Rats Subjected to Different Treatments

Only a set of data was obtained in left and down spinal cord in right sciatic nerve transection and spinal cord transection group (RSNTASCT/LD), so this group was not performed with different comparison. The results of RT-PCR showed that, in the simple sciatic nerve transection group, the BDNF mRNA expression in the left spinal cord (0.79±0.10) and right spinal cord (0.84±0.54) were lower than that in the sham group (0.87±0.14), the P value are 0.248 and 0.614 which were greater than 0.05, respectively. The BDNF mRNA expression in the left and up spinal cord in the right sciatic nerve transection and spinal cord transection group (RSNTASCT/LU) (0.81±0.11), in the right and up spinal cord in the right sciatic nerve transection and spinal cord transection group (RSNTASCT/RU) (0.86±0.06) are lower than that in the sham group (0.87±0.14), the P value are 0.303 and 0.766, respectively. The BDNF mRNA expression in the right and down spinal cord in the right sciatic nerve transection and spinal cord transection group (RSNTASCT/RD) (0.99±0.13) is higher than that in sham group, the P value is 0.152. The BDNF mRNA expression in the right spinal cord in the simple right sciatic nerve transection group (RSNT/RS) (0.84±0.05), in the RSNTASCT/LU (0.81±0.11) and in the RSNTASCT/RU (0.86±0.06) were higher than that in the left spinal cord in the simple right sciatic nerve transection group (RSNT/LS) (0.79±0.10), the P value are 0.528, 0.850 and 0.377, respectively. However, right sciatic nerve transection and spinal cord transection led to the obvious increasing of BDNF mRNA expression in the right and down spinal cord (0.99±0.13), compared with that in the RSNT/LS (0.79±0.10), the P value is 0.023. The BDNF mRNA expression in the RSNTASCT/LU (0.81±0.11) is lower than that in the RSNT/RS (0.84±0.05), the P value is 0.634. The BDNF mRNA expression in the RSNTASCT/RU (0.86±0.06) and the RSNTASCT/RD (0.99±0.13) were higher than that in the RSNT/RS (0.84±0.05), the P value are

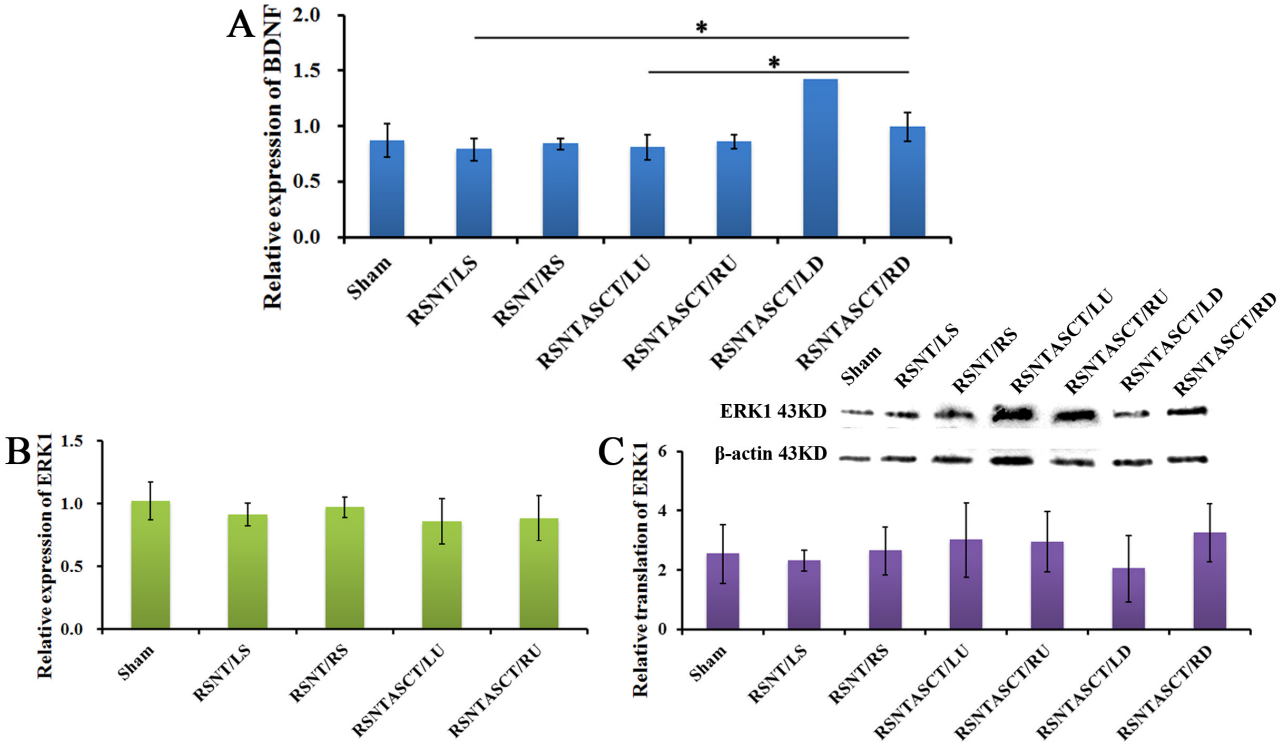

Figure 1 BDNF mRNA, ERK1 mRNA and protein expression in the spinal cord of rats subjected to different treatments. *(A) The BDNF mRNA expression in the spinal cord of rats subjected to different treatment. (B) The ERK1 mRNA expression in spinal cord of rats subjected to different treatment. (C) The ERK1 protein expression in spinal cord of rats subjected to different treatment. Sham: normal spinal cord. RSNT/LS: left spinal cord in simple right sciatic nerve transection group. RSNT/RS: right spinal cord in simple right sciatic nerve transection group. RSNTASCT/LU: left and up spinal cord in right sciatic nerve transection and spinal cord transection group. RSNTASCT/RU: right and up spinal cord in right sciatic nerve transection and spinal cord transection group. RSNTASCT/LD: left and down spinal cord in right sciatic nerve transection and spinal cord transection group. RSNTASCT/RD: right and down spinal cord in right sciatic nerve transection and spinal cord transection group.*

0.822 and 0.075, respectively. The BDNF mRNA expression in the RSNTASCT/RU (0.86±0.06) is higher than that in the RSNTASCT/LU (0.81±0.11), the P value is 0.459 . However, the BDNF mRNA expression in the RSNTASCT/RD (0.99±0.13) is higher than that in the RSNTASCT/LU (0.81±0.11), the P value is 0.026. The BDNF mRNA expression in the RSNTASCT/RD (0.99±0.13) is higher than that in the RSNTASCT/RU (0.86±0.06), the P value is 0.095. Detailed information is shown in Fig.1A.

The Comparison of ERK1 mRNA in Spinal Cord of Rats Subjected to Different Treatment

The results of RT-PCR indicated that the ERK1 mRNA expression in the left spinal cord in the simple right sciatic nerve transection group (RSNT/LS) (0.91±0.09), in the right spinal cord in the simple right sciatic nerve transection group (RSNT/RS) (0.97±0.08), in left and up spinal cord in the right sciatic nerve transection and spinal cord transection (RSNTASCT/LU) (0.86±0.18) and in the right and up spinal cord in the right sciatic nerve transection and spinal cord transection (RSNTASCT/RU) (0.88±0.18) are lower than that in sham group (1.02±0.15), the P value are 0.219, 0.547, 0.078 and 0.140repectively. The ERK1 mRNA expression in the RSNT/RS (0.97±0.08) is higher than that in the RSNT/LS (0.91±0.09), the P value is 0.518. Nevertheless, the ERK1 mRNA expression in the RSNTASCT/LU (0.86±0.18) and the RSNTASCT/RU (0.88±0.18) are lower than that in the RSNT/LS (0.91±0.09), the P value are 0.562 and 0.736, respectively. The ERK1 mRNA expression in the RSNTASCT/LU (0.86±0.18) and the RSNTASCT/RU (0.88±0.18) are lower than that in the RSNT/RS (0.97±0.08), the P value are 0.227 and 0.348, respectively. The ERK1 mRNA expression in the RSNTASCT/RU (0.88±0.18) is higher than that in the RSNTASCT/LU (0.86±0.18), the P value is 0.833. Detailed information is described in Fig.1B.

The Comparison of ERK1 protein in Spinal Cord of Rats Subjected to Different Treatment

The results of western blot demonstrated that the ERK1 protein expression in the RSNT/LS (2.32±0.36) and the RSNTASCT/LD (2.05±1.12) are lower than that in sham group (2.54±0.98), the P value are 0.746 and 0.510, respectively. However, the ERK1 protein expression in the RSNT/RS (2.65±0.81), in the RSNTASCT/LU (3.01±1.25), in the RSNTASCT/RU (2.95±1.01) and the RSNTASCT/RD (3.25±0.98) are higher than that in the sham group (2.54±0.98), the P value are 0.876, 0.500, 0.552 and 0.304, respectively. The ERK1 protein expression in the RSNT/RS (2.65±0.81),

in the RSNTASCT/LU (3.01±1.25), in the RSNTASCT/RU (2.95±1.01) and the RSNTASCT/RD (3.25±0.98) are higher than that in the RSNT/LS (2.32±0.36), the P value are 0.632, 0.322, 0.362 and 0.182, respectively. The ERK1 protein expression in the RSNTASCT/LD (2.05±1.12) is lower than that in the RSNT/LS (2.32±0.36), the P value is 0.718. The ERK1 protein expression in the RSNTASCT/LU (3.01±1.25), in the RSNTASCT/RU (2.95±1.01) and the RSNTASCT/RD (3.25±0.98) are higher than that in the RSNT/RS (2.65±0.81), the P value are 0.604, 0.660 and 0.381, respectively. The ERK1 protein expression in the RSNTASCT/LD (2.05±1.12) is lower than that in the RSNT/RS (2.65±0.81), the P value is 0.423. The ERK1 protein expression in the RSNTASCT/RU (2.95±1.01) and the RSNTASCT/LD (2.05±1.12) are lower than that in the RSNTASCT/LU (3.01±1.25), the P value are 0.936 and 0.206, respectively. However, the ERK1 protein expression in the RSNTASCT/RD (3.25±0.98) is higher than that in the RSNTASCT/LU (3.01±1.25), the P value is 0.716. The ERK1 protein expression in the RSNTASCT/LD (2.05±1.12) is lower than that in the RSNTASCT/RU (2.95±1.01), the P value is 0.233. However, the ERK1 protein expression in the RSNTASCT/RD (3.25±0.98) is higher than that in the RSNTASCT/RU (2.95±1.01), the P value is 0.657. The ERK1 protein expression in the RSNTASCT/RD (3.25±0.98) is higher than that in the RSNTASCT/LD (2.05±1.12), the P value is 0.115. Detail is shown in Fig.1C.

The Comparison of BDNF mRNA in Cortex of Rats Subjected to Different Treatments

The results of RT-PCR showed that the BDNF mRNA expression in the left cortex in the simple right sciatic nerve transection group (RSNT/LC) (0.83±0.11), in the right cortex in the simple right sciatic nerve transection group (RSNT/RC) (0.85±0.08), in left cortex in the right sciatic nerve transection and spinal cord transection group (RSNTASCT/LC) (0.96±0.20) and the right cortex in the right sciatic nerve transection and spinal cord transection group (RSNTASCT/RC) are higher than that in the sham group (0.77±0.06), the P value are 0.542, 0.470, 0.095 and 0.158, respectively. The BDNF mRNA expression in the RSNT/RC (0.85±0.08), in the RSNTASCT/LC (0.96±0.20) and the RSNTASCT/RC (0.92±0.10) are higher than that in the RSNT/LC (0.83±0.11), the P value are 0.860, 0.148 and 0.271, respectively. The BDNF mRNA expression in the RSNTASCT/LC (0.96±0.20) and the RSNTASCT/RC (0.92±0.10) are higher than that in the RSNT/RC (0.85±0.08), the P value are 0.221 and 0.384, respectively. The BDNF mRNA expression in the RSNTASCT/RC (0.92±0.10) is lower than that in the RSNTASCT/LC (0.96±0.20), the P value is 0.660 that is greater than 0.05, so there is no statistical significance.

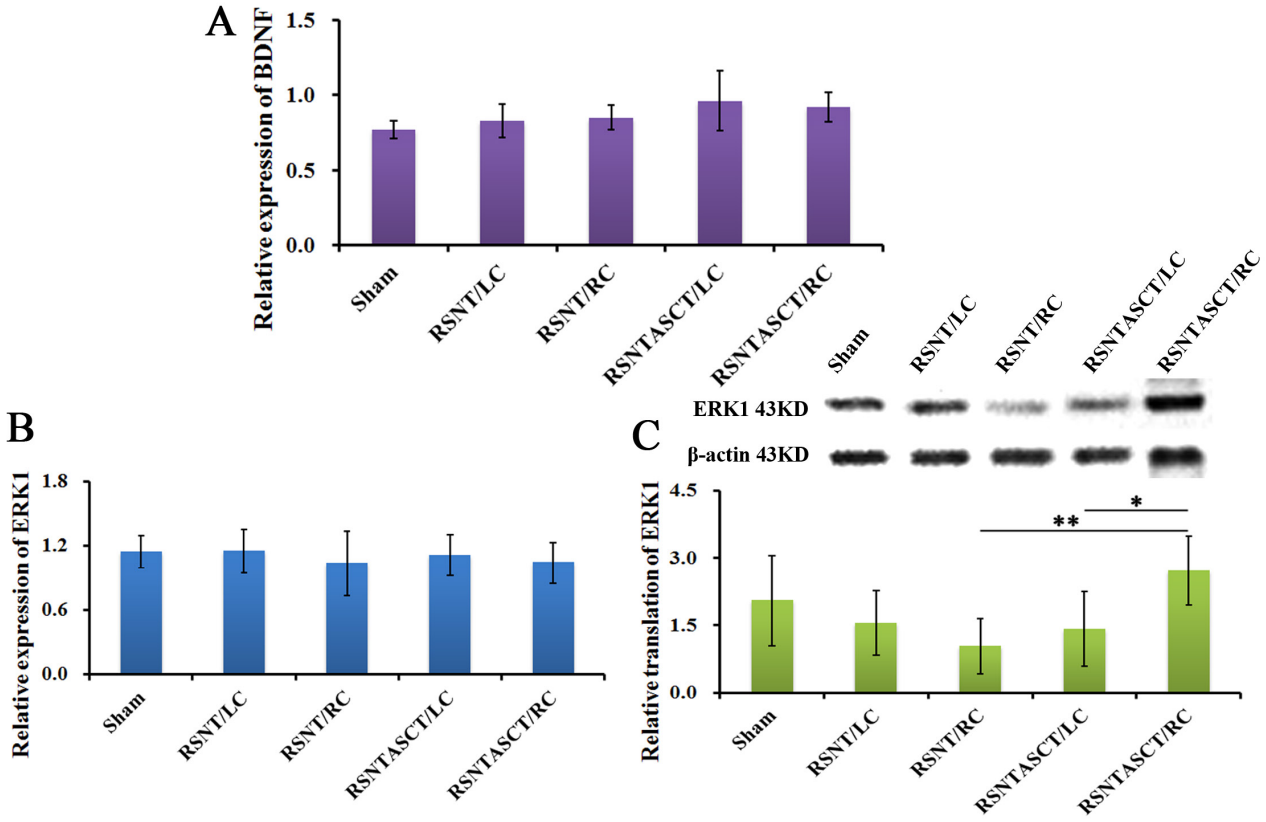

Fig.2 BDNF mRNA, ERK1 mRNA and ERK1 protein expression in the cortex of rats subjected to different treatments. *A The BDNF mRNA expression in the cortex of rats subjected to different treatments. Sham: normal cortex. B The ERK1 mRNA expression in the cortex of rats subjected to different treatments. C The ERK1 protein expression in the cortex of rats subjected to different treatment. Sham: normal cortex. RSNT/LC: left cortex in the simple right sciatic nerve transection group. RSNT/RC: right cortex in the simple right sciatic nerve transection group. RSNTASCT/LC: left cortex in the right sciatic nerve transection and spinal cord transection group. RSNTASCT/RC: right cortex in the right sciatic nerve transection and spinal cord transection group.*

Detailed information is shown in Fig. 2A.

The Comparison of ERK1 mRNA in Cortex of Rats Subjected to Different Treatments

The results of RT-PCR indicated that the ERK1 mRNA expression in the RSNT/LC (1.15±0.20) was higher than that in the sham group (1.14±0.15), the P value is 0.907. The ERK1 mRNA expression in the RSNT/RC (1.03±0.30), in the RSNTASCT/LC (1.11±0.19) and the RSNTASCT/RC (1.04±0.19) are lower than that in the sham group (1.14±0.15), the P value are 0.474, 0.835 and 0.484, respectively. The ERK1 mRNA expression in the RSNT/RC (1.03±0.30), in the RSNTASCT/LC (1.11±0.19) and the RSNTASCT/RC (1.04±0.19) are lower than that in the RSNT/LC (1.15±0.20), the P value are 0.385, 0.730 and 0.389, respectively. The ERK1 mRNA expression in the RSNTASCT/LC (1.11±0.19) and the RSNTASCT/RC (1.04±0.19) are higher than that in the RSNT/RC (1.03±0.30), the P value are 0.583 and 0.955, respectively. The ERK1 mRNA expression in the RSNTASCT/RC (1.04±0.19) is lower than that in the RSNTASCT/LC (1.11±0.19), the P value is 0.601. Detail information is described in Fig.2B.

The Comparison of ERK1 protein in Cortex of Rats Subjected to Different Treatments

The results of western blot demonstrated that the ERK1 protein expression in the RSNT/LC (1.55±0.72), in the RSNT/RC (1.03±0.61) and the RSNTASCT/LC (1.42±0.83) are lower than that in the sham group (2.05±1.00), the P value are 0.376, 0.079 and 0.271, respectively. However, the ERK1 protein expression in the RSNTASCT/RC (2.72±0.77) is higher than that in the sham group (2.05±1.00), the P value is 0.237. The ERK1 protein expression in the RSNT/RC (1.03±0.61) and the RSNTASCT/LC (1.42±0.83) are lower than that in the RSNT/LC (1.55±0.72), the P value are 0.374 and 0.831, respectively However, the ERK1 protein expression in the RSNTASCT/RC (2.72±0.77) is higher than that in the RSNT/LC (1.55±0.72), the P value is 0.06. The ERK1 protein expression in the RSNTASCT/LC (1.42±0.83) is higher than that in the RSNT/RC (1.03±0.61), the P value is 0.496. However, the ERK1 protein expression in the RSNTASCT/RC (2.72±0.77) is higher than that in the RSNT/RC (1.03±0.61), the P value is 0.01. The ERK1 protein expression in the RSNTASCT/RC (2.72±0.77) is higher than that in the RSNTASCT/LC (1.42±0.83), the P value is 0.039.

Discussion

In this study, we built a neural tramatic model via sciatic nerve transection and spinal cord transection, which could contribute to a series of neurologic symptoms. Meanwhile, numerous factors and moleculars will change accompanying with the stress reaction and repairmen of body. Wonderfully, we serendipitously found the expression of BDNF was obviously changed. In addition, the level of ERK1 was also altered. Our finding is the first time to show the change of BDNF and ERK1 after nervous system injury. These results implied that self-healing capabilities of tissue may derive from certain cytokines. Therefore, we speculate that BDNF play a role associated with ERK1 pathway after undergoing conditioning leison in the spinal cord or cortex. This is an innovative discovery, which maybe conducive to exploit a noval therapeutic target for clinic treatment. Whereas, the undelying mechanism of this progress may refer to multiple genes expression. Regretfully, because of the limitations of Technology and funds, we concentrate only in the superficial phenomenons and could not point out what are the reasons can account for this progress. Consequently, in order to further clarify the mechanism, greater works will be performed in the future.

The pivotal role of BDNF in the nervous system

In our research, neurological deficits were induced by sciatic nerve transection and spinal cord transection,which leading to a severe deterioration in motor function. Previously, we found BDNF changed dramatically utilizing RT-PCR and Western Blotting technologies. BDNF is a member of the neurotrophin family which plays a crucial role in nervous system development and function and it is is involved in the pathogenesis of a wide range of nervous system diseases (Ikegame T, et al., 2013). BDNF can promote neural differentiation, survival of nerve cells, neurite outgrowth, and synaptic plasticity. Meanwhile, it is widely expressed throughout the mammalian brain, including the cerebral cortex, hippocampus, basal forebrain, striatum, hypothalamus, brainstem, limbic structures and cerebellum (Murer MG, et al., 2001). BDNF structure and another family of neurotrophic factors in the structure of nerve growth factor are the residues of 50% homology, contains six cysteine residues, three tryptophan residues and aspartic acid residue, linked by disulfide bond between them, maintain the structure and biological functions play an important role of BDNF. Studies have shown that neurons activity closely associated with BDNF secretion, its development in the process could promote neurons and expression of neuronal development, growth, survival and function.

The pivotal role of ERK in nervous system diseases, and the possible regulatory relationships between BDNF and ERK

The current study has demonstrated that extracellular signal-regulated kinase (ERK) is essential for normal development and functional plasticity of the central nervous system. Moreover, a growing number of recent studies in models of cerebral ischemia, brain trauma and neurodegenerative diseases imply a crucial role for ERK1/2 signaling during neuronal injury (Chu CT, et al., 2004). ERK is a versatile protein kinase that regulates many cellular functions. Growing evidence indicates that ERK1 pathway plays a key role in promoting cell survival, proliferation and apoptosis in a variety of neuronal systems(Subramaniam S, et al., 2010). Recent papers stated briefly that ERK1/2 translocation to the nucleus and the proteins involved in the cytosolic retention of activated ERK1/2, further studies have confirmed that ERK1/2 pathway is pivotal for enhancing cell survival and death, so it is essential to harness this pathway for developing effective treatments for neurological disorders (Mebratu Y, et al., 2009). Chronic neurodegenerative diseases, increased ERK1/2 phosphorylation has been noted in the vulnerable penumbra following acute ischemic stroke

in humans (Slevin M, et al., 2000). Many reports showed clearly ERK1/2 signaling pathway may contribute to acute nervous system injuries (Alessandrini A, et al., 1999), and plentiful studies have ensued that further substantiated the neuroprotective effect of ERK1/2 in neuronal cell lines and primary neuron cultures (Hetman M, et al., 2004). ERK1/2 signalling pathway promotes cell survival and apoptosis via a dual mechanism comprising the posttranslational modification and inactivation of a component of the cell death machinery and the increased transcription of pro-survival genes (Kolch W. 2005), which involve in many factors, such as BDNF.

Xie Y et al found that BDNF modulation through the ERK1/2 pathway under the acute stress (Xie Y, et al., 2013). Xiaolong Wang et al showed that the expression of BDNF was significantly lower, however, the level of phosphorylated ERK1/2 was higher. BDNF is known to protect against neuronal loss caused after nervous damage (Mattson MP, et al., 2004), which along with their downstream signaling mediated by extracellular, signal-regulated Erk1/2 (Atif F, et al., 2013). Franco JL also exhibited that ERK phosphorylation and BDNF expression in the cerebral cortex were increased after antidepressants treatment (Franco JL, et al., 2008). Hence, we hypothesis that up-regulation of BDNF may be related to the ERK pathway to exert neuroprotective effects, but this will require lots of additional works to be performed.

Conclusion

In summary, spinal cord will restrain the level of BDNF, and sciatic nerve transection can promote ERK1 expression. Whereas, right sciatic nerve transection and spinal cord transection will promote ERK1 expression. At the same time, right sciatic nerve transection and spinal cord transection will promote BDNF and ERK1 expression, but sciatic nerve transection can suppress ERK1 expression in cortex.

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Conflits of interest

All authors declare that they have no conflict of interests.

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