Effects of Epidural Spinal Cord Stimulation and Treadmill Training on Locomotion Function and Ultrastructure of Spinal Cord Anterior Horn after Moderate Spinal Cord Injury in Rats*

WANG Yizhao[1] HUANG Xiaolin[1,3] XU Jiang[1] XU Tao[1] FANG Zhengyu[1]
XU Qi[2] TU Xikai[2] YANG Peipei[2]

Abstract **Objective:** To investigate the effects of epidural spinal cord stimulation (ESCS) and treadmill training on the locomotion function and ultrastructure of spinal cord anterior horn after moderate spinal cord injury in rats. **Method:** Nine adult female Sprague-Dawley rats were randomly distributed into three groups: ①spinal cord injury group (SI, n=3). ②spinal cord injury plus ESCS group (SE, n=3). ③spinal cord injury plus treadmill training group (TT, n=3). All rats received a moderate spinal cord injury surgery. Four weeks after surgery, rats in SE group received an electrode implantation procedure, with the electrode field covering spinal cord segments L2—S1. Four weeks after electrode implantation, rats received subthreshold ESCS for 30 min/d. Rats in TT group received 4cm/s treadmill training for 30min/d. Rats in SI group received no intervention, as a control group. All procedures in these three groups lasted four weeks.The open field Basso,Beattie and Bresnahan (BBB) scale was used before and after intervention to evaluate rats′ hindlimb motor function. **Result:** After four weeks intervention, rats in TT group improved their open field locomotion scores to 20. In contrast, no significant improvement was observed in groups SI and SE. The morphology of synapses and neurons were similar regardless of whether rats had undergone ESCS, treadmill training or not. **Conclusion:** ESCS alone was not sufficient to improve the walking ability of spinal cord injured rats. ESCS or treadmill training alone might not contribute to the changes of ultrastructure in anterior horn of spinal cord that underlie the recovery of walking ability. Further research is needed to understand the contributions of combination of ESCS and treadmill training to the rehabilitation of spinal cord injured rats.

Key words spinal cord stimulation; anterior horn; ultrastructure; spinal cord injury; rats

Applications of pharmacon, epidural spinal cord stimulation (ESCS), ambulation training, have been used successfully to promote functional recovery in animal experiments [1—3]. Partial weight bearing therapy followed by ESCS could facilitate functional walking in chronic incomplete spinal cord injured patient [4—5]. These results might be due to the reinforcement of specific sensorimotor pathways resulting in more selective and stable network of neurons that control locomotion. Nevertheless, researches on the mechanism of these interventions focused on electrophysiology [4,6—8], and histology of spinal cord [1]. In contrast, the effects of these interventions applied respectively or jointly on ultrastructures of spinal cord gray substance, are still not well understood. In the present investigation, we examined the effects of ESCS and treadmill training on the locomotion function and ultrastructure of spinal cord anterior horn in rats with moderate spinal cord injury.

1 MATERIAL AND METHOD

1.1 Material

The experimental procedures were complied with the institutional guidelines of Tongji Medical College of Huazhong University of Science and Technology. All surgical procedures were performed under aseptic conditions. Nine adult female Sprague-Dawley rats (200—250g body weight) were used in this experiment. Animals were randomly divided into three groups: ①spinal cord injury group (SI, n=3), ②spinal cord injury plus ESCS group (SE, n=3), ③spinal cord injury plus treadmill training group (TT, n=3).

1.2 Method

1.2.1 Spinal cord injury procedure: Partial laminectomies were performed at the T8 level after anesthetized with chloral hydrate (300mg/kg, i.p.) [9]. Every rat received moderate severe contusive injury at vertebral level T8 using a weight-drop device striking the spinal cord with a 10g

*This study was supported by the National Natural Science Foundation of China with grant No. 60874035 and Tongji Hospital Research Fund with grant No. 2008013.

1 Department of Rehabilitation Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China

2 Key Laboratory of Image Processing and Intelligent Control, Department of Control Science and Technology, Huazhong University of Science and Technology, Wuhan 430000, China

3 Corresponding Author: Xiaolin Huang(xiaolinh2006@yahoo.com.cn)

weight dropped from a height of 5 centimeters. After the incision sites were sutured, all rats were housed individually, and the bladders of the spinal cord injured rats were expressed manually two times per day. Hindlimbs of all rats were moved passively through a full range of motion once daily to maintain joint function.

1.2.2 ESCS electrode implantation procedure: The rats in SE group underwent electrode implantation procedures four weeks after spinal cord injury. After anesthesia, the incision and laminectomy made four weeks ago was exposed again. An electrode was inserted through the dorsal gap of epidural space to make sure that the electrode field covered the spinal cord at segments L2—S1. Wings of the electrode were sutured to the paravertebral fascia and muscle to warrant the electrode setting in epidural space statically. Two wires connecting the argyric contactors of electrode were fixed at the back surface of rats. All rats were housed individually after the incisions were closed, with intensive post surgical care.

Stimulating electrodes were made of polyimide to guarantee biocompatibility [10—11]. A suitably thickness (0.139 mm) and width (1.42mm) can avoid compressioning to the spinal cord. Electric current was spread between two round argyric contactors (diameter 1.22mm, 15.00mm gap between two contactors) at the front end of electrode contacting the spinal cord. A self-made electrical stimulator could generate electric pulses as needed.

1.2.3 Treadmill device: A treadmill device was custom-made for rats in this study, with work velocities of 0—15mm/s.

1.2.4 ESCS and treadmill training schedule: The rats in SE group received daily subthreshold ESCS after electrode implantation (200mV voltage, 200μs pulse width, 40Hz frequency)[6—8] for 30 min. Rats in TT group received 4cm/s treadmill training for 30min/d. The rats in SI group received no intervention as a control group. All procedures in these three groups lasted four weeks. The open field Basso, Beattie and Bresnahan (BBB) scale was used before and after the intervention (at the week 9—12, once a week) to evaluate hindlimb function[12].

1.2.5 Transmission electron microscopy: All rats in these three groups were deeply anesthetized with chloral hydrate (i.p.) and perfused with 4% paraformaldehyde after four weeks of intervention at the week 12. Their L2 spinal cord segments were removed, and 1 mm^3 tissue of anterior horn was cut and fixed immediately in 3% glutaraldehyde. These pieces were embedded following standard protocols and double-stained with uranyl acetate and lead citrate for electron microscopy [13]. Ultrathin sections were imaged on a Tecnai G2 12 transmission electron microscope (FEI Company, The Netherlands). Finally, the ultrastructures of synapses and neurons in anterior horn of spinal cord were examined.

1.3 Statistical analysis

All the data were presented as mean ± SE. BBB Scale scores of different groups recorded every weekly were compared using one-way repeated measures ANOVA. Differences were considered significant at $P \leq 0.05$.

2 RESULT

2.1 BBB Scale scores

BBB open field locomotion scores were assessed once a week to evaluate hindlimb function, before and after the period of intervention. All rats showed normal locomotion before spinal cord injury, obtaining a maximum score in the neuroethology evaluation. All rats′ hindlimb function reduced to an extremely low score after spinal cord injury procedure. All rats scored close to 0 immediately after the procedure, and their scores increasing to a moderate score of 9 eight weeks later. There was no significant difference among three groups at this stage. At week 9 to week 12, after four weeks intervention, a statistic improvement to a score of 20 appeared in TT group. Neither SI nor SE group showed significant improvement, and there was no significant differences between these two groups. Importantly, improvement in TT group was significantly different from SI and SE groups at this stage (Fig.1).

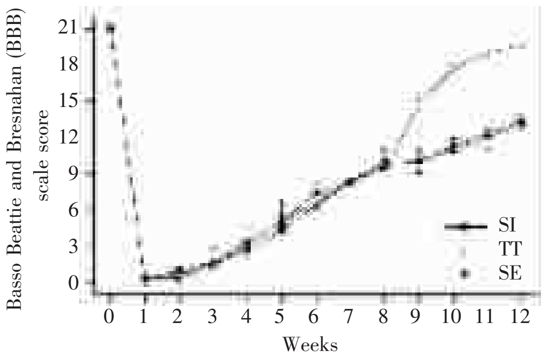

Fig. 1 BBB scale scores of three groups during 12 weeks of the experiment

Group TT obtained significantly higher scores than groups SI and SE after 12 weeks

2.2 Ultrastructural observation of synapses and neurons

Whether the rats underwent ESCS and treadmill training or not, synapses had similar gross morphology (Fig. 2), and there was no significant difference in synapses among these three groups. There was no difference in neurons among these three groups also. The nucleus, mitochondria and the rough endoplasmic reticulum were nicely shaped, with no swelling. It suggested that neurons in anterior horn of spinal cord were normal regardless of whether the rats underwent ESCS and treadmill training or not (Fig. 3).

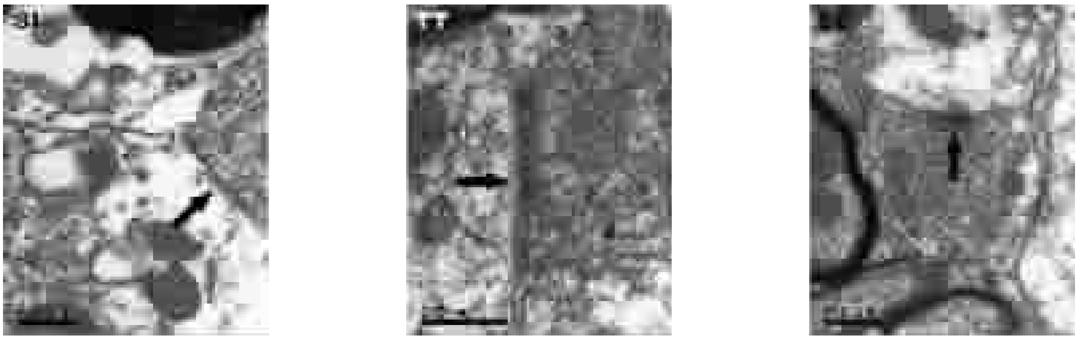

Fig. 2 Ultrastructural features of synapses in anterior horn of spinal cord

(solid arrow: synapse)

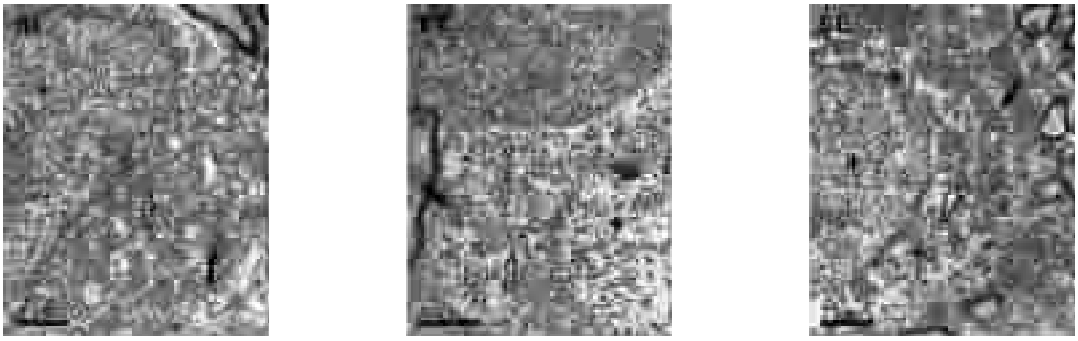

Fig. 3 Ultrastructural features of neurons in anterior horn of spinal cord

(*: nucleus; solid arrow: rough endoplasmic reticulum; hollow arrow: mitochondria)

3 DISCUSSION

There were clinical and animal experiments reporting the contribution of ESCS to ambulation recovery of subjects with spinal cord injuries [4,6]. Herman et al. discovered that conditioned ESCS on lumbar enlargement of an incomplete cervical spinal cord injury patient resulted in less sense of effort in walk[5]. Lavrov et al. found that ESCS can facilitate rat′s locomotion after spinal cord lesion, in a time-dependent manner correlated with long-latency responses to ESCS[6]. In contrast to these reports in the present study, we found no improvement in rats of SE group, which might be due to the fact that ESCS just stimulated the lumbar enlargement, but not along with step training as described in the reports. These results suggest that application of ESCS combined with treadmill training may be important for ESCS′s effectiveness.

Treadmill training is commonly used to help paraplegic animals or patients regaining walking ability [14—17]. Heng and DeLeon [15] discovered that treadmill training could restore normal patterns of hindlimb movements following severe incomplete spinal cord injury in rats, which was consistent with our results.

However, we have not observed any significant difference in synapses among these three groups of animals. The shape, length, and number of synapses were hardly changed under ESCS or treadmill training. Goldshmit Y et al. used treadmill training after spinal cord hemisection in mice,and found no axonal regeneration into or across the lesion site, indicating that the improved behaviour may be, at least in part, due to enhanced neural activity above the lesion site[18]. Tissues detected in our experiment were below the lesion site, thus leading to no significant change of synapse. Likewise, subthreshold ESCS may fail to influence synapses involved in the neural pathways in spinal cord anterior horn. Neurons in anterior horn seemed similar within each of the three groups of animals, also indicating that ESCS and treadmill training might contribute to the changes in the spinal cord pathways underlying the recovery of ambulation in other way. However, ESCS combined with step training can indeed help paraplegic animals or patients regaining walking ability [5—6], so further experiments focusing on combination of ESCS and treadmill training are needed to explore these unclear mechanisms.

In conclusion, treadmill training could improve the rat′s hindlimb loconotion,but ESCS alone was not sufficient to improve the walking ability of rats with spinal cord lesions. This could be due to the fact that ESCS were not applied along with ambulation training. Thus, combination of ESCS and treadmill training may be necessary for ambulation recovery. Neurons and synapses within the anterior horn were not different among these three groups of rats, indicating that ESCS and treadmill training alone might not contribute to the changes in neuronal pathways in the spinal cord anterior horn that underlie the recovery of walking ability. Further research is needed to understand the contributions of combination of ESCS and treadmill training to the rehabilitation of spinal cord injury rats.

REFERENCE

[1] Ichiyama RM, Courtine G, Gerasimenko YP, et al. Step training reinforces specific spinal locomotor circuitry in adult spinal rats[J]. J Neurosci, 2008, 28(29): 7370—7375.

[2] Edgerton VR, Kim SJ, Ichiyama RM, et al. Rehabilitative therapies after spinal cord injury [J]. J Neurotrauma, 2006. 23 (3—4): 560—570.

[3] Gerasimenko YP, Ichiyama RM, Lavrov IA, et al. Epidural spinal cord stimulation plus quipazine administration enable stepping in complete spinal adult rats[J]. J Neurophysiol, 2007. 98(5): 2525—2536.

[4] Carhart MR, He J, Herman R, et al. Epidural spinal-cord stimulation facilitates recovery of functional walking following incomplete spinal-cord injury [J]. IEEE Trans Neural Syst Rehabil Eng, 2004, 12(1): 32—42.

[5] Herman R, He J, D'Luzansky S, et al. Spinal cord stimulation facilitates functional walking in a chronic, incomplete spinal cord injured[J]. Spinal Cord, 2002, 40(2): 65—68.

[6] Lavrov I, Dy CJ, Fong AJ, et al. Epidural stimulation induced modulation of spinal locomotor networks in adult spinal rats[J]. J Neurosci, 2008, 28(23): 6022—6029.

[7] Gerasimenko YP, Lavrov IA, Courtine G, et al. Spinal cord reflexes induced by epidural spinal cord stimulation in normal awake rats[J]. J Neurosci Methods, 2006, 157(2): 253—263.

[8] Ichiyama RM, Gerasimenko YP, Zhong H,et al. Hindlimb stepping movements in complete spinal rats induced by epidural spinal cord stimulation [J]. Neurosci Lett, 2005, 383 (3): 339—344.

[9] Streng T, Hedlund P, Talo A, et al. Phasic non-micturition contractions in the bladder of the anaesthetized and awake rat [J]. BJU Int, 2006, 97(5): 1094—1101.

[10] Yeager JD, Phillips DJ, Rector DM, et al. Characterization of flexible ECoG electrode arrays for chronic recording in awake rats [J]. J Neurosci Methods, 2008, 173(2): 279—285.

[11] Lago N, Yoshida K, Koch KP, et al. Assessment of biocompatibility of chronically implanted polyimide and platinum intrafascicular electrodes [J]. IEEE Trans Biomed Eng, 2007, 54 (2): 281—290.

[12] Goldshmit Y, Lythgo N, Galea MP, et al. Treadmill training after spinal cord hemisection in mice promotes axonal sprouting and synapse formation and improves motor recovery [J]. J Neurotrauma, 2008, 25(5): 449—465.

[13] Zheng M, Ruan Y, Yang M, et al. The comparative study on ultrastructure and immunohistochemistry in AFP negative and positive hepatocellular carcinoma [J]. J Huazhong Univ Sci Technolog Med Sci, 2004, 24(6): 547—549, 559.

[14] Cai LL, Fong AJ, Otoshi CK, et al. Implications of assist-as-needed robotic step training after a complete spinal cord injury on intrinsic strategies of motor learning [J]. J Neurosci, 2006, 26(41): 10564—10568.

[15] Heng C, de Leon RD. Treadmill training enhances the recovery of normal stepping patterns in spinal cord contused rats [J]. Exp Neurol, 2009, 216(1): 139—147.

[16] Gorassini MA, Norton JA, Nevett-Duchcherer J,et al. Changes in locomotor muscle activity after treadmill training in subjects with incomplete spinal cord injury [J]. J Neurophysiol, 2009, 101(2): 969—979.

[17] Mehrholz J, Kugler J, Pohl M. Locomotor training for walking after spinal cord injury [J]. Spine, 2008, 33(21): E768—777.

[18] Goldshmit Y, Lythgo N, Galea MP, et al. Treadmill training after spinal cord hemisection in mice promotes axonal sprouting and synapse formation and improves motor recovery [J]. J Neurotrauma, 2008, 25(5): 449—465.