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Electrical stimulation promotes regeneration of injured oculomotor nerves in dogs

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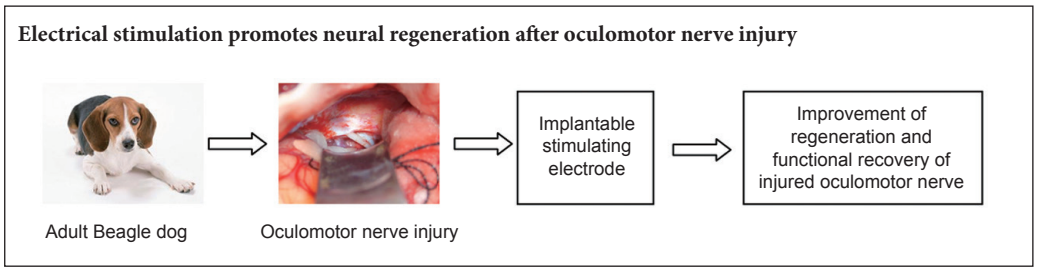
Graphical Abstract



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Abstract

Functional recovery after oculomotor nerve injury is very poor. Electrical stimulation has been shown to promote regeneration of injured nerves. We hypothesized that electrical stimulation would improve the functional recovery of injured oculomotor nerves. Oculomotor nerve injury models were created by crushing the right oculomotor nerves of adult dogs. Stimulating electrodes were positioned in both proximal and distal locations of the lesion, and non-continuous rectangular, biphasic current pulses (0.7 V, 5 Hz) were administered 1 hour daily for 2 consecutive weeks. Analysis of the results showed that electrophysiological and morphological recovery of the injured oculomotor nerve was enhanced, indicating that electrical stimulation improved neural regeneration. Thus, this therapy has the potential to promote the recovery of oculomotor nerve dysfunction.

Key Words: *nerve regeneration; oculomotor nerve; electrical stimulation; dog; nerve injury; model; cranial nerve; peripheral nerve*

Introduction

Oculomotor nerve dysfunction is usually associated with severe craniocerebral trauma or surgery on the base of the skull (Flanders et al., 2012; Gu et al., 2012; Lin et al., 2013). Conventional treatment of oculomotor nerve injury includes medication and surgical decompression. Unfortunately, the prognosis of oculomotor nerve injury is very poor, and the mechanism underlying the regeneration of injured oculomotor nerves is still unclear (Kim and Chang, 2013; Lin et al., 2013; Zhu et al., 2013). Recently, electrical stimulation was used to accelerate peripheral nerve regeneration in both animal experiments and clinical practice (Haastert-Talini and Grothe, 2013; Kao et al., 2013; Zhang et al., 2013; Kuffler, 2014; Suszynski et al., 2015). The purpose of the present study was to investigate whether non-continuous electrical stimulation could enhance the regeneration of injured oculomotor nerves and improve their functional recovery in dogs.

Materials and Methods

Animals

Sixteen female adult Beagles (age 24 months; weight 9.0–11.0 kg) were obtained from the Animal Research Center of Shanghai Jiao Tong University (Permits SCXK (Hu) 20070004). The 16 dogs were randomly allotted into injury (control) and injury with electrical stimulation (stimulation) groups (n = 8 per group). Animals were housed in separate cages. Experiments were performed and animals were cared for in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of China (The Ministry of Science and Technology of the People's Republic of China, 1988). The study was approved by the Ethics Committee for Animal Care and Use, Shanghai Jiao Tong University.

Establishment of an oculomotor nerve injury model

General anesthesia was achieved *via* intramuscular injection

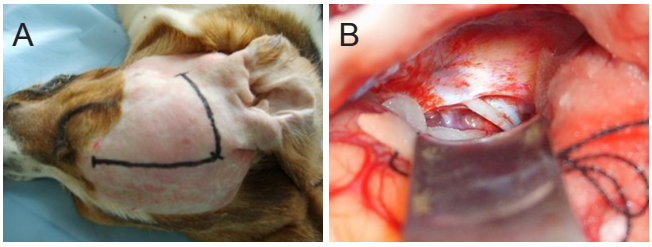

Figure 1 Establishment of an oculomotor nerve injury model.
(A) A U-shaped incision was made just above the midpoint of the superciliary arch, extending straight towards the ear, turning ventrally just in front of the auricle, and reaching the posterior extremity of the zygomatic arch. (B) The right oculomotor nerve (black arrow) was crushed in the cistern segment between the midbrain and the cavernous sinus in all dogs.

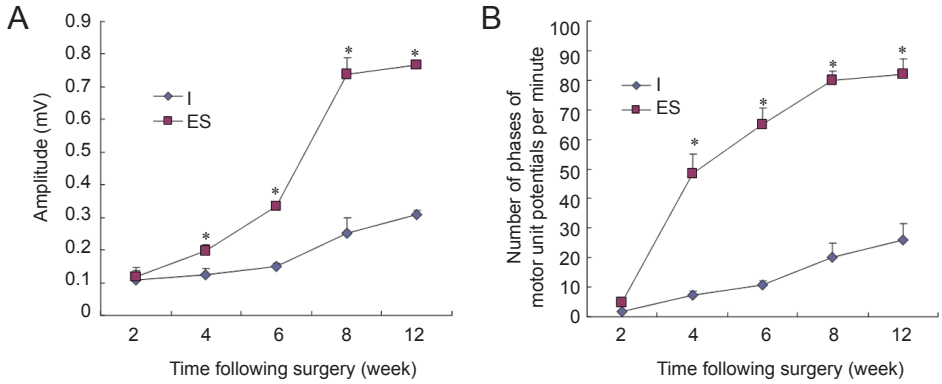

Figure 3 Effect of stimulation on the electrophysiology of damaged oculomotor nerves.
(A) Mean amplitude of motor unit potentials. (B) Mean number of phases in the motor unit potentials. Data are expressed as the mean ± SEM. Differences between stimulation and control groups were assessed using the two-sample *t* test. **P* < 0.05, *vs.* injury group. ES: Electrical stimulation group; I: injury group.

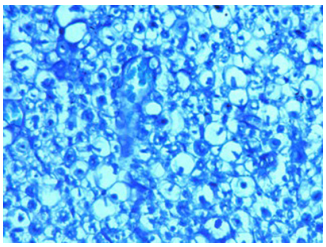

Figure 2 Effect of electrical stimulation on the histology of damaged oculomotor nerves (toluidine blue staining, light microscope, × 40).
An injured nerve in the stimulation group.

of a mixture of ketamine (10 mg/kg, Jiangsu Hengrui Medicine Co., Ltd., Jiangsu, China), diazepam (1 mg/kg, Shanghai Xudonghaipu Pharmaceuticals Co., Ltd., Shanghai, China) and atropine (0.05 mg/kg, Shanghai Hefeng Pharmaceuticals Co., Ltd., Shanghai, China). Atropine was administered to inhibit saliva secretion.

A right-modified pterional approach was adopted (**Figure 1**A), and the cistern segments of the right oculomotor nerve were exposed in all dogs after craniotomy (Zhu et al., 2013). The portion of each right oculomotor nerve located between its exit from the midbrain and its entrance into the cavernous sinus was crushed by complete occlusion for 30 seconds using a gun-shaped forceps (**Figure 1B**).

Electrical stimulation

Implantable stimulating electrodes that we designed (Patent No. CN201299648, Shanghai, China) were encircled proximally and distally around the trunk of the nerve relative to the injured site. The two de-insulated electrodes were fixed onto the muscle with suture, allowing the current from the stimulator to pass through the crushed tissue. The electrode leads were routed subcutaneously to the scalp, where the electrodes were secured on the temporal surface of the skull. Additionally, a needle electrode used for recording was placed in the inferior oblique muscle. Incisions were closed and the dogs were taken back to their cages and allowed to recover after surgery (Wang et al., 2012).

In the stimulation group, dogs received direct stimulation with non-continuous, rectangular, 20-ms bipolar current pulses per phase (Powerlab System, AD Instruments Pty Ltd., Castle Hill, Australia) at a frequency of 5 Hz, 1 hour per day for 2 consecutive weeks. The security and stability of the implanted electrodes were inspected each week throughout the experiment.

Electromyography examination

The Powerlab system was employed for oculomotor nerve stimulation and electromyographic (EMG) examination. All dogs received spontaneous EMG examination 2, 4, 6, 8, and 12 weeks after surgery to assess the regulation of functional muscle reinnervation. Motor unit potentials (MUPs) were recorded when audio- or light-induced ocular movement occurred in conscious animals. The amplitude and phase number of MUPs were recorded and compared. For all dogs, MUPs of the inferior obliquus, eyeball movement, pupil diameter, and light reflex were monitored regularly during the experimental period.

Histological changes in the injured oculomotor nerve

Twelve weeks after surgery, dogs were anesthetized with ketamine, diazepam, and atropine and sacrificed. Afterwards, the oculomotor nerve (1.0 cm in length) was dissected and sections were fixed in formaldehyde, stained with toluidine blue, rinsed, dried, and fixed and sealed with neutral balsam. Morphological changes were observed using light microscopy (Olympus, Tokyo, Japan).

Statistical analysis

Data are expressed as the mean ± SEM. Statistical analysis was performed using SPSS 16.0 software (SPSS, Chicago, IL, USA). Intergroup differences were compared with the two-sample *t* test. $P < 0.05$ was considered statistically significant.

Results

Effect of electrical stimulation on the functional recovery of damaged oculomotor nerves

All dogs completed the experiment. After the right oculomotor nerve was crushed, mydriasis, absence of papillary light reflex, ptosis, and eyeball-movement dysfunction were detected immediately. In the injury group, pupil diameter was slightly reduced, and the indirect and direct pupillary light reflex were absent in only one dog at 2 weeks post-surgery. Miosis occurred in three dogs at 8 weeks. All dogs suffered from eyeball-movement impairment at 12 weeks. In contrast, three dogs in the stimulation group presented with significantly smaller pupil diameters at 2 weeks, and the direct pupillary light reflex had recovered in two dogs by 6 weeks post-surgery. Importantly, eyeball movement had improved significantly in five dogs by 12 weeks, while only three dogs remained without any improvement.

Effect of electrical stimulation on the histological changes of damaged oculomotor nerves

Toluidine blue staining showed disordered oculomotor nerve fibers and thin myelin sheaths in the stimulation group at 12 weeks after surgery (**Figure 2**).

Effect of electrical stimulation on the electrophysiology of damaged oculomotor nerves

At 4, 6, 8, and 12 weeks following surgery, MUP amplitude of the oculomotor nerves was significantly higher in the stimulation group than that in the control group ($P < 0.05$; **Figure 3A**). Additionally, the number of phases was significantly higher in the stimulation group than that in the control group ($P < 0.05$; **Figure 3B**).

Discussion

Peripheral nerve regeneration is a complex process, including bidirectional interactions between regenerated axons and targets (Goodman and Bercovich, 2013; Kuffler, 2014). Because regeneration of the oculomotor nerve is so difficult, many surgeons and researchers have thought that its regeneration is almost impossible (Fernandez et al., 1997). However, with the development of micro-neurosurgery techniques, neurosurgeons have gained better insight into this issue (Sekhar et al., 1992; Yang et al., 2011; Zhu et al., 2013). Previous studies have shown that the degree of functional recovery attained after oculomotor nerve injury primarily depends on the number of remaining oculomotor neurons and their axons (Fernandez et al., 1997; Yang et al., 2011; Zhu et al., 2013). However, little is known about the molecular mechanisms underlying the regeneration of axons, midbrain motor neurons, or electrophysiological changes (Fernandez et al., 1997). Recent studies have suggested that electrical stimulation can be used to accelerate some peripheral nerve regeneration following nerve injury (Haastert-Talini and Grothe, 2013; Zhang et al., 2013). However, until the current study, this method had not yet been applied to the oculomotor nerve. Likely reasons for this are that the implanted stimulating electrodes had failed to stay fixed to other cranial nerves and that the electrical stimulation parameters were difficult to determine. Addtionally, we analyzed the histological changes in oculomotor nerve fibers after oculomotor nerve injury. This study demonstrated the beneficial effects of electrical stimulation on canine oculomotor nerve regeneration.

Typically, evoked extraocular muscle activity is used to monitor ocular motor nerve function (Liang et al., 2012). Indeed, electrophysiological monitoring is becoming a very important method for evaluating nerve function (Zhou et al., 2012). In the present study we analyzed EMG recordings in the inferior obliquus, which is considered an appropriate tool for evaluating functional recovery of injured oculomotor nerves. Our method of chronically stimulating the oculomotor nerve enhanced its regeneration. After 2 weeks of chronic electrical stimulation, spontaneous MUP amplitudes in the stimulation group showed considerably more improvement than they did in the control group. The amplitude of inferior oblique MUPs in the control group were very low at 2, 4, and 6 weeks after surgery, although they had significantly increased by 8 weeks. In contrast, significantly higher amplitudes were observed at almost all periods in the stimulation group. Additionally, compared with the control group, pupil diameter was lower and the pupillary light reflex was partially recovered in the electrical stimulation group. What we observed was consistent with previous studies, in that the recovery of the pupillary light reflex was earlier than other functions (Fernandez et al., 1987; Pallini et al., 1992)

Activated regeneration of the oculomotor nerve in dogs has been observed in the distal nerve segments (Yang et al., 2011) after the main trunk was crushed. For example, Wallerian degeneration and revascularization was ongoing, with Wallerian degeneration of the distal axons beginning and macrophages entering the damaged area to remove the myelin and axonal debris on day 3 after operation (Fansa et al., 2001). During this process, the basement membranes surrounding the axon and the Schwann cells remain intact. Schwann cells concentrate in the basement membrane tubes and synthesize growth factors, which attract axonal sprouts formed at the terminal of the proximal segment of the severed axon. The basement membrane tubes provide pathways for the regenerated axons to connect muscles and skin. The Schwann cells then remyelinate the newly formed axons. However, the newly formed myelin is thinner than normal and the newly formed internodes are shorter than normal. Moreover, our findings are consistent with previously reported in vivo peripheral nerve studies using chronic electrical stimulation (Hegarty and Goroszeniuk, 2011; Haastert-Talini and Grothe, 2013).

Inevitably, the pathophysiological mechanism still needs

further research. Our results can answer the question regarding the time course of recovery after the oculomotor nerve was partially injured. We also find that the mechanism by which the oculomotor nerve regenerates might be associated not only with electrophysiology of midbrain motor neurons, but also with physiological changes in the damaged nerve trunks.

In conclusion, our study identified positive effects of electrical stimulation on the regeneration of the oculomotor nerve and recovery of motor function. Electrical stimulation might therefore be a potential therapy for promoting the recovery of oculomotor nerve dysfunction. Electrical stimulation has been proposed as a therapeutic approach to enhance the speed and specificity of axonal regeneration after nerve injury (Geremia et al., 2007). Based on the present study, we can recommend that future research should focus on this technique for injured cranial nerve regeneration.

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