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Effects of Unilateral Electroacupuncture on Bilateral Proprioception in a Unilateral Anterior Cruciate Ligament Injury Model

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Background: Anterior cruciate ligament (ACL) injury can cause knee proprioception degeneration, on which the electroacupuncture (EA) treatment has a definite effect. However, it is still not clear whether conducting EA intervention on the injured side can promote bilateral proprioception recovery.

Material/Methods: We randomly selected 6 of 9 normal cynomolgus monkeys to develop unilateral ACL injury models via arthroscopy. All knees were divided into 5 groups: the normal control (NC) group, injured side of blank model (ISBM) group, contralateral side of blank model (CSBM) group, injured side of EA (ISE) group, and contralateral side of EA (CSE) group. Ten days after modeling, the monkeys in the EA group were treated with EA daily for 6 weeks at the acupoints. At 6 weeks, the 5 groups were examined by electrophysiology (SEPs and MCV). The ACL was separated to conduct the gold chloride staining for morphology observation and count the number of total and variant proprioceptors.

Results: At 6 weeks, the latent period of the SEPs and MCV and the number of variant proprioceptors in the blank model group and the EA group were increased compared with the NC group, while the amplitude and the number of total proprioceptors were decreased. The changes in the ISBM and CSBM group were more remarkable than in the ISE and CSE group. All differences were statistically significant ($P<0.05$).

Conclusions: Unilateral ACL injury leads to bilateral proprioception degeneration, and the unilateral knee EA intervention can aid bilateral proprioception recovery.

MeSH Keywords: **Anterior Cruciate Ligament • Electroacupuncture • Proprioception**

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Background

Anterior cruciate ligament (ACL) injury, which occurs mostly in young people, is the most common ligament injury in the United States. It is reported that more than 200 000 people suffer from ACL damage every year in the United States [1] and the number continues to increase in both the general population and in people who play sports. As an important stabilizing structure of the knee joint, ACL not only directly provides static mechanical stabilization, but also has a neural proprioceptive afferent function and maintains the dynamic stability of the knee joint [2–4]. Therefore, ACL injury may lead to knee joint dysfunction, instability, and weak legs due to impaired biomechanics. Degeneration of knee joint proprioception function can also reduce dynamic stability, leading to meniscus, cartilage injury and muscle atrophy, increasing the prevalence of knee osteoarthritis, and affecting the activity and stability of the joint [5–7], which is a vicious circle. It is internationally recognized that ACL injury can impair biomechanics and proprioception and reduce the stability of the knee joint.

ACL damage can lead to degeneration of proprioception on the injured side and the contralateral side [8–10]. Clinically, for individuals who have undergone unilateral ACL reconstruction, a subsequent rupture of the contralateral ACL is very troublesome. According to a recent meta-analysis, ACL injury in patients with knee proprioception was significantly lower than that of normal people, and the injured side was significantly lower than that of the contralateral, while after reconstruction, the proprioception was significantly better than preoperatively [11]. The rate of the contralateral ACL injury, which occurs typically 1–4 years after an index ACL reconstruction, was reported to range from 2% to 15% [12], and the rate of subsequent contralateral reconstruction was reported to vary with the length of follow-up. Some studies have found unilateral ACL injuries were followed up for 5 years after a reconstruction, and nearly 4.6–9% of the patients needed a contralateral ACL reconstruction [13–15]. A study by Andermord showed that 270 participants (3.0%) of a total of 9061 participants underwent primary contralateral ACL reconstruction during the 5-year follow-up (age <20 years significantly increased the risk of contralateral reconstruction) [16]. It was also found that even though the injured ACL is histologically reconstructed and its physical stability has been fundamentally improved, many patients still have instability of the knee joint [17,18]. To date, the presence of proprioception deficit and the extent of its recovery after an ACL reconstruction have not been conclusively established. However, it is known that with the extension of the reconstruction time, the degree of postoperative recovery improves [19–21].

To promote the recovery of proprioception after an ACL injury, many methods have been proposed depending on the severity of ACL injury, which is commonly divided into 3 types: mild, moderate, and severe. In general, it is mainly treated by conservative or surgical treatment. In terms of the severity of injury, ligament reconstruction can be selected. In addition, other treatments such as acupuncture, biofeedback training and support, and vibration balance training are also important methods of proprioception rehabilitation. Conservative treatment is the main method for degrees I and II and is done by pressure bandaging, ice compress, external application of Chinese medicine, acupuncture, and massage. Oakes cut off the ACLs of sheep, used conservative treatment, and regularly observed their recovery [22], finding that the ligament healed after 3 years and had a high toughness and elasticity, which was strong evidence supporting use of conservative treatment. Surgical treatment is the main treatment for grades III and IV, and ACL reconstruction with stump preservation is the most widely used procedure.

However, even though the ligament is reconstructed and the PNF is used, proprioception often is compromised for a long time and the recovery time is not clear [17,18]. EA can be used for conservative treatment after an acute ACL injury, as well as for the recovery of proprioception after a reconstruction. Some studies have shown that when ACL is stimulated by electricity, the related flexor or extensor muscle can be activated reflexively and participate in stabilizing activities of the knee joint, which can also be maintained by the reflex activities of ligaments and muscles [23–25]. Once the stability of the knee joint is strengthened, proprioception can be recovered and ACL re-injury prevented. Han et al. confirmed that EA stimulation therapy had a definite effect on the treatment of pain, movement disturbance, substance dependence, and tissue regeneration [26–28], and numerous studies have shown that EA intervention can prevent the subsequent degeneration of proprioception of the knee joint.

ACL injury, which can cause knee proprioception degeneration, is very common. Even though the injured ACL is histologically reconstructed, there may still be instability of the knee joint; therefore, research on this topic is important. There is no doubt that EA intervention can aid proprioception. However, it is still not clear whether it can promote proprioceptive recovery when conducting EA intervention on the injured side. Therefore, this study aimed to investigate the effects of unilateral EA on bilateral proprioception.

Material and Methods

Ethical statement

All the procedures were approved by the Ethics Inspection Committee of Animal Experiments of Yunnan Yingmore

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Biological Technology Co., Ltd. (No. YBT1602). The welfare of animals was guaranteed by the Association for Assessment and Accreditation of Laboratory Animal Care International and animal care was performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (Office of Science and Health Reports CPRR/NIH 1996).

Experimental animal and feeding

Nine specific pathogen-free (SPF) male cynomolgus monkeys were purpose-bred by and purchased from Yunnan Yingmore Biological Technology Co., Ltd, with accreditation of animal research facilities by Association for Assessment and Accreditation of Laboratory Animal Care International. All monkeys were housed at the Laboratory Animals Breeding Center of Yunnan Yingmore Biological Technology Co.

Grouping and modeling

According to random number table method, 6 of the 9 cynomolgus monkeys were chosen for developing the model of unilateral ACL injury, then 3 of them underwent the EA intervention, while the remaining 3 were assigned to the NC group. Model cynomolgus monkeys were treated by unilateral ACL injury, while cynomolgus monkeys in the NC group were not. All the knee joints were divided into 5 groups: the NC group (3 normal knees were chosen from 3 normal cynomolgus monkeys, 1 knee per monkey), the ISBM group (3 injured knees from 3 monkeys that were developed as models), the CSBM group (3 contralateral knees in 3 monkeys that were developed as models), the ISE group (3 injured knees in 3 monkeys that were treated with EA intervention), and the CSE group (3 contralateral knees in 3 monkeys that were treated with EA intervention).

Developing models and methods

We used Zoletil 50 anesthetics to anaesthetize the monkeys by intramuscular injection. After anesthetization, we kept these model animals in the supine position and made an incision through the anteromedial and anterolateral approach of the knee joint using arthroscopic surgery, probing the articular cartilage of knee, ACL, and meniscus. After confirming these tissues were intact, we then severed 1 in 4 sections of the ACL by bush-hook. We washed the articular cavity and incision with normal physiological saline. Finally, we sutured the incision so that it would heal within 10 days. There were no infections or delayed healing.

EA intervention

For the EA group, we select acupoints named Wei Yang (in the Bladder Meridian of Foot-taiyang), Yin Gu (in the Kidney Meridian of Foot-Shaoyin), Xi Yangguan (in the Gallbladder Meridian of Foot-Shaoyang), and Qu Quan (in the Liver Meridian of Foot-Jueyin), which are all located in the hamstring (including semitendinosus, semimembranosus, and biceps femoris muscles) (Figure 1). When EA was stimulated, these acupoints cause muscle contraction of the hamstring. We pierced these acupoints for about 15 min with filiform needles and performed EA treatment with an acupoint nerve stimulator at a frequency of 2/100 Hz and an intensity of 3 mA for 15 min once daily for 6 weeks for a course of treatment. The filiform needles (0.3×40 mm) used in the treatment were purchased from Chinese Suzhou Medical Supplies Factory Co., Ltd. We used an acupoint nerve stimulator (model: G91-D, Yangzhou Kangling Medical Electronic Instrument Co. Ltd.) and evoked potential meter (model: MEB-9402C, Japan optoelectronic company). The NC group and the blank model group did not undergo EA intervention.

Figure 1. Acupuncture points on the injured side of the knee.

Nerve electrophysiology and histopathology

After 6 weeks, 3 knee joints from each group were chosen from each of the 5 groups, then the changes in ACL nerves were assessed by electrophysiology, somatosensory evoked potentials (SEPs), and motor nerve conduction velocity (MCV). SEPs and MCV were assessed by 2 methods of monitoring of peripheral nerve injury, which mainly have 2 indicators: the latent period and the amplitude. Extended latent period and decreased amplitude indicate nerve injury. As a control, the same electrophysiological inspections were performed on the monkeys in the blank model group after group division. (1) SEPs: The SEPs at room temperature (26–28°C) were measured, and the skin, which was the corresponding position of both sides of the ACL attached area by bipolar surface electrode, was

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Table 1. The comparison of the latent period and amplitude in 5 groups (mean ±SD).

Groups	SEPs		MCV	
	Latent period (ms)	Amplitude (μV)	Latent period(ms)	Amplitude (mV)
ISBC	25.87±0.62[abc]	2.43±0.06[abc]	18.73±0.32[abc]	1.43±0.15[abc]
CSBC	16.70±0.66[bc]	4.33±0.15[bc]	10.53±0.25[bc]	2.43±0.21[bc]
ISE	19.43±0.68[bc]	3.50±0.26[bc]	14.47±0.95[bc]	1.97±0.21[bc]
CSE	14.03±0.57[c]	5.47±0.15[c]	5.80±0.87[c]	8.20±0.26[c]
NC	11.97±0.21	7.57±0.21	3.12±0.03	9.53±0.42

ISBM – injured side of blank model; CSBM – contralateral side of blank model; ISE – injured side of electroacupuncture; CSE – contralateral side of electroacupuncture; NC – normal control. [a] P<0.05 *vs.* the CSBM group; [b] P<0.05 *vs.* the same side of electroacupuncture group; [c] P<0.05 *vs.* the NC group.

stimulated. The stimulative parameter was constant voltage (single square wave electrical stimulation, wave width 0.1 ms, stimulation intensity 15 to 20 mA). Then cynomolgus monkeys' SEPs, measured by photoelectric evoked potentiometer (MEB-9402C), were recorded, and the information was input to a microcomputer operation system. Finally, the graphics latent period and amplitude indexes of SEPs were measured and analyzed. (2) MCV: The stimulating electrode was placed at the popliteal space for stimulation, the recording electrode was placed at the hamstring muscle belly, and the reference electrode was placed at the recording electrode by 2 cm at room temperature (26–28°C). Then, the skin, which is the corresponding position of both sides of the ACL attached area by bipolar surface electrode, was stimulated. The stimulative parameter was constant voltage (single square wave electrical stimulation, wave width 0.1 ms, frequency 2 Hz, stimulation intensity 25 to 30 mA). Cynomolgus monkeys' SEPs were recorded by photoelectric evoked potentiometer (MEB-9402C) and the waveform and latent period of MCV were measured and analyzed.

Gold chloride staining

After the inspection of SEPs and MCV, under euthanasia, ACLs of all monkeys were obtained completely and put into the miscible liquids of lemon juice and 88% formic acid (lemon juice: 88% formic acid=3: 1) in preparation for staining. Firstly, all specimens were placed for 15 min in a dark room. Secondly, l% gold chloride solutions were added for 30 min and 25% formic acid solution for 15 h, washed by distilled water for 1 h, and the pure glycerin was added for 24 h, in that order. After staining, tissue blocks were dehydrated in an ascending series of ethanol, cleared in xylene, and embedded in paraffin. Then, 5-μm sections were prepared using a rotary microtome (Leica, CM3050S, Germany). Five sections were randomly chosen from each paraffin block of ACL to represent all parts of the ACL as far as possible. Finally, parts of the femur, tibia, and the intermediate location were marked by 3 operators, each of whom counted the number of proprioceptors in ACLs and hamstrings, as well as the surrounding tissues, to avoid duplication.

Statistical analysis

All data are presented as mean ±SD. Groups were compared using one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test in SPSS version 20.0. Differences between groups were considered significant at p<0.05.

Results

Nerve electrophysiology

At 6 weeks, compared with the NC group, the latent period of the SEPs and MCV of the blank model group and the EA group increased, while the amplitude significantly decreased. Meanwhile, compared with the ISBM and CSBM group, the latent period of SEPs and MCV were extended in the ISE and CSE group, respectively, while the amplitude of SEPs and MCV decreased. Changes in the CSBM group were better than in the ISE group (P<0.05). Intervention with EA significantly reduced the latent period and increased the amplitude of the SEPs and MCV (P<0.05) (Table 1).

Histopathology

At 6 weeks, compared with the NC group, the number of total proprioceptor decreased, but the number of variant proprioceptor increased in the ISBM, CSBM, ISE, and CSE groups (P<0.05). Meanwhile, the number of total proprioceptor in the ISBM and CSBM group had significantly decreased in comparison with the ISE and CSE group, but the number of variant proprioceptors in the ISBM and CSBM groups had significantly increased in comparison with the ISE and CSE group, and the

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Table 2. The comparison of the number of total and variant proprioceptor in 5 groups (mean ±SD).

Groups	ISBC	CSBC	ISE	CSE	NC
Total (N)	587.33±9.71[abc]	802.00±15.13[bc]	701.00±11.53[bc]	891.00±14.42[c]	976.00±6.00
Variant (N)	37.67±2.52[abc]	9.33±0.58[bc]	25.67±2.08[bc]	4.33±0.58[c]	0.00±0.00

ISBM – injured side of blank model; CSBM – contralateral side of blank model; ISE – injured side of electroacupuncture; CSE – contralateral side of electroacupuncture; NC – normal control. [a] $P<0.05$ *vs.* the CSBM group; [b] $P<0.05$ *vs.* the same side of electroacupuncture group; [c] $P<0.05$ *vs.* the NC group.

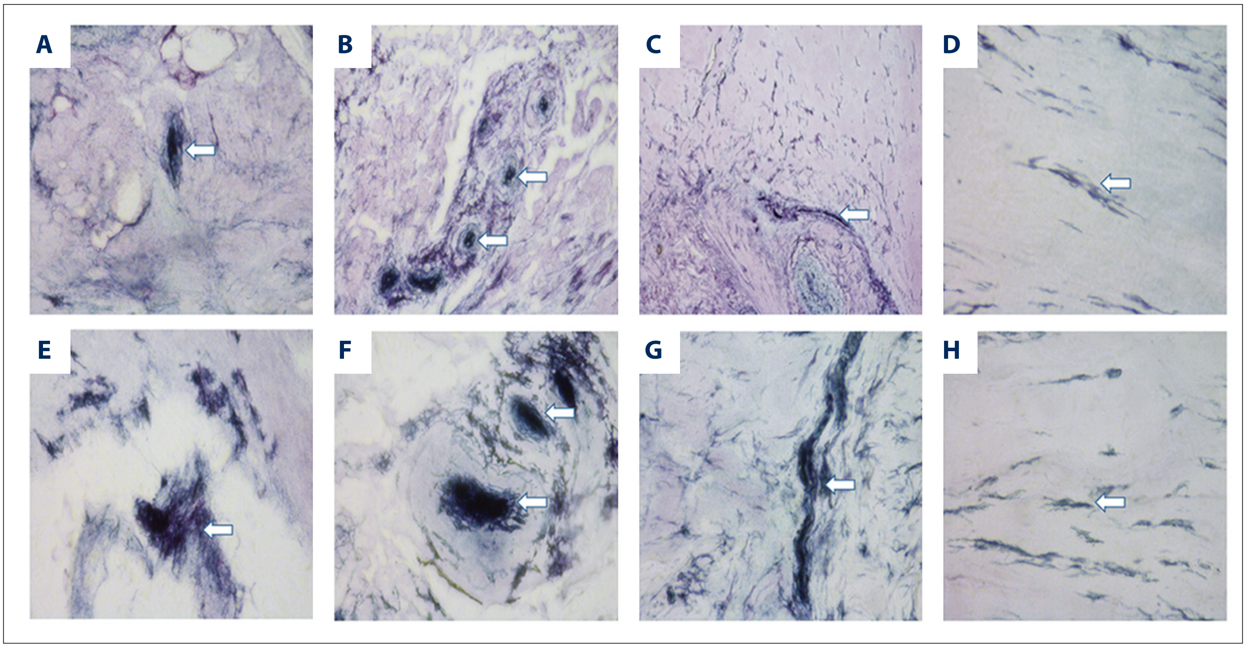

Figure 2. The pathological morphology changes of normal and variant proprioceptors (Original magnification: ×400). The places where the arrows point to are the locations of the proprioceptors. The upper row is the mutated, while the lower is the normal. The arrows in **A–C** from the left to the right are variant Ruffini corpuscle, variant Pacinian corpuscle, and variant Golgi tendon organs, respectively; (**D**) Free nerve endings in the injured ACL, where there are fewer nerve endings; (**E–H**) Ruffini corpuscle, Pacinian corpuscle, Golgi tendon organs, and free nerve endings in the normal ACL, respectively.

CSBM group was worse than the ISE group ($P<0.05$). EA intervention obviously slowed the reduction rate of the number of total proprioceptors ($P<0.05$) and affected the number of variant proprioceptors ($P>0.05$) (Table 2, Figure 2).

Discussion

As for the grouping selection, many clinical studies have confirmed that unilaterally ACL injury can lead to a decrease in bilateral proprioception of the knee. However, most of them just contrasted the injured side with the uninjured, instead of dealing with the opposite side. Therefore, we suggest that the normal knee joint should be chosen as the control rather than the uninjured side.

It is accepted that EA treatment of proprioception degeneration caused by ACL injury has a definite effect. Most studies have selected some patients with isolated ACL injury who underwent ACL reconstruction to conduct EA treatment or electrical stimulation, then passive angle reproduction test method was used to assess the sense of position, the stability, and tension of the graft by use of an KT 2000 instrument, while the postoperative knee joint function is estimated by the Lysholm scale and IKDC scale. As a result, the subjectivity of all of the results is strong [25,29–31]. In our study, subjective evaluations were not used. Cynomolgus monkeys that possess similar human gene and have the same way of walking were chosen to develop ACL injury animal models. After EA intervention, we used physiological method and gold chloride staining, and observed the morphology and quantity of proprioceptors changes to evaluate nerve and ACL proprioceptors injury. Thus, our results are more intuitive and reliable. Adachi has found a positive

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correlation between the number of mechanoreceptors and accuracy of the joint position sense [32], and our study aimed to observe changes in the basic shape of ligament and the physiology, morphology, and quantity of proprioceptors after EA intervention on the knee joint of unilateral ACLs. Acupoints Wei Yang, Yin Gu, Xi Yangguan, and Qu Quan were chosen for the EA group among many acupoints because they are the main points around the knee joint in the treatment of knee joint pain or difficulty in extension and flexion. We performed acupuncture on the points along the local meridian around the knee joint, taking advantage of the proximal treatment's effect, stimulating the peripheral nerve by electroacupuncture, achieving the regeneration and rehabilitation of nerve, then activating the flexor and extensor tendons reflexively. The knee joint activities were stabilized and the function of the proprioception of the joint were reconstructed.

Some studies have also found that the unilateral ACL injury can lead to proprioception degeneration of the bilateral knee joint, indicating that the proprioception nerve conduction of the bilateral limbs has a cross-connection, which belongs to the same ganglia that jointly control it [12,33]. Because of this characteristic, we can damage or interfere with proprioception of one limb, which will not only affect the injury or intervention side, but also the proprioceptive sense of the contralateral limb, to cure the contralateral side or the bilateral sides. This finding is full of clinical meaning, for its provides a new idea for treatment, especially when the contralateral side is badly injured and hard to treat while the other side is uninjured and easy to treat. In our results, at 6 weeks, compared with the NC group, the number of total proprioceptors and the amplitude of the SEPs and MCV decreased, while the number of variant proprioceptors and the latent period of the SEPs and MCV increased in the ISBM, CSBM, ISE, and CSE groups ($P<0.05$). This phenomenon indicated that unilateral ACL injury could lead to bilateral proprioception degeneration of the knee joint. What's more, compared with the ISE and CSE group, the latent period of SEPs and MCV were extended, while the number of proprioceptors and the amplitudes of SEPs and MCV decreased in the ISBM and CSBM groups respectively, and the changes in the CSBM group were better than in the ISE group ($P<0.05$), indicating that unilateral EA intervention can produce a positive effect on the bilateral proprioception recovery. It has been proved that upward neurons of proprioception at different levels receive bilateral limb nerve conduction with the interactive connection [33–35], which may be why bilateral EA intervention can have a bilateral cross-treatment effect, letting us achieve better results via the bilateral knee EA intervention model.

Limitations of this study are that there were few experimental animals and all of them were males, making it hard to evaluate whether the sex of model animal had an effect on the experiment. Secondly, the study was limited to 6 weeks and the long-term follow-up changes of proprioception could not be observed.

Conclusions

Unilateral ACL injury leads to bilateral proprioception degeneration, while unilateral knee EA intervention can aid bilateral proprioception recovery, and the effects on the injured side are better.

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Conflict of interests

None.

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