# Microelectronic neural bridging of toad nerves to restore leg function☆

## Xiaoyan Shen[1, 2], Zhigong Wang[2], Xiaoying Lv[3], Zonghao Huang[2]

1 School of Electronic Information, Nantong University, Nantong 226007, Jiangsu Province, China
2 Institute of RF- & OE-ICs, Southeast University, Nanjing 210096, Jiangsu Province, China
3 State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096, Jiangsu Province, China

## Abstract

The present study used a microelectronic neural bridge comprised of electrode arrays for neural signal detection, functional electrical stimulation, and a microelectronic circuit including signal amplifying, processing, and functional electrical stimulation to bridge two separate nerves, and to restore the lost function of one nerve. The left leg of one spinal toad was subjected to external mechanical stimulation and functional electrical stimulation driving. The function of the left leg of one spinal toad was regenerated to the corresponding leg of another spinal toad using a microelectronic neural bridge. Oscilloscope tracings showed that the electromyographic signals from controlled spinal toads were generated by neural signals that controlled the spinal toad, and there was a delay between signals. This study demonstrates that microelectronic neural bridging can be used to restore neural function between different injured nerves.

Xiaoyan Shen☆, M.D., Associate professor.

Corresponding author: Zhigong Wang, M.D., Professor, Institute of RF- & OE-ICs, Southeast University, Nantong 210096, Jiangsu Province, China, zgwang@seu.edu.cn.

## Research Highlights

(1) A microelectronic neural bridge was built comprising electrode arrays for neural signal detection, functional electrical stimulation, and a microelectronic circuit.
(2) The spinal toad animal model does not inhibit generation and transmission of neural signals or inhibit neural signals generated by functional electrical stimulation. The entire body can maintain biological activity free of control by the brain.
(3) With the help of microelectronic neural bridge, the function of the left leg of one spinal toad can restore the function on the corresponding leg of another spinal toad.

## INTRODUCTION

The rebuilding of lost motor and sensory functions, caused by an injury or disease of the nervous system, has emerged as one of the most important and challenging tasks in clinical neuroscience[1-3]. The rapid growth and development at the intersection of neuroscience, electronics, and engineering has led to the creation of revolutionary neurotechnologies that can regenerate lost neural function[4-7]. Functional electrical stimulation can be used to restore motor function in individuals with paralysis[8-9]. Current functional electrical stimulation systems provide muscle activation that is controlled by external electrical impulse signals that restore useful movements[10-12]. Reawakening of the spinal networks below a

lesion can be accomplished through electrical stimulation[13-15].

A common feature of functional electrical stimulation methods mentioned above is that pulse patterns are artificially formed[16-18]. Alternatively, lost neural function of an injured nerve can be restored by means of a microelectronic neural bridge, which bridges the two ends of an injured nerve without artificial pulse patterns.

The concept of a microelectronic neural bridge was proposed in 2005[19-21]. Several microelectronic neural bridge modules have been implemented in the form of hybrid- and monolithically-integrated circuits, and a series of animal experiments carried out. Using 50 rats, it has been demonstrated that the neural signal from the proximal stump of the sciatic nerve or spinal cord can be regenerated in the distal stump of the sciatic nerve or spinal cord. In rabbits, it has been shown that an interrupted spinal cord can be bridged bidirectionally when two microelectronic neural bridge systems are inserted between the controlling and the stimulated nerve in two directions. Both externally evoked and spontaneously generated neural signals have been successfully regenerated. Relevant reflexes of the legs and related neural signal waveforms have been observed. Thus, the feasibility of the microelectronic neural bridge method has been demonstrated[22].

Experiments with rats and rabbits, however, have two limitations: (1) a degree of anaesthesia is necessary for electrode positioning. Though anaesthesia can maintain the rats or rabbits in a quiescent state, it greatly inhibits the activity of the nervous system; (2) the source of neural signals of microelectronic neural bridge are evoked by means of external electrical stimuli. Consequently, only simple reactions such as twitches or shakes are observed.

To overcome these limitations in this study, we used spinal toads whose brains were destroyed, but with their reflex arc intact. Furthermore, sciatic signals associated with a movement without any stimulation artefact were extracted and used as the source signal for microelectronic neural bridge. As movement is controlled by a neural signal[23], only if the microelectronic neural bridge extracts and transmits the sciatic signal successfully can movement, similar to a withdrawal reflex, be regenerated when the signal is acting on the controlled spinal toad, even without external stimulation. The present study aimed to verify the possibility of restoring leg movement using microelectronic neural bridge between the legs of two separate toads with

mechanical or chemical stimulation.

## RESULTS

### Quantitative analysis of animals
A total of 250 toads were used and included in the final analysis.

### Restoration of leg function *via* sciatic nerves of different toads using mechanical stimulation
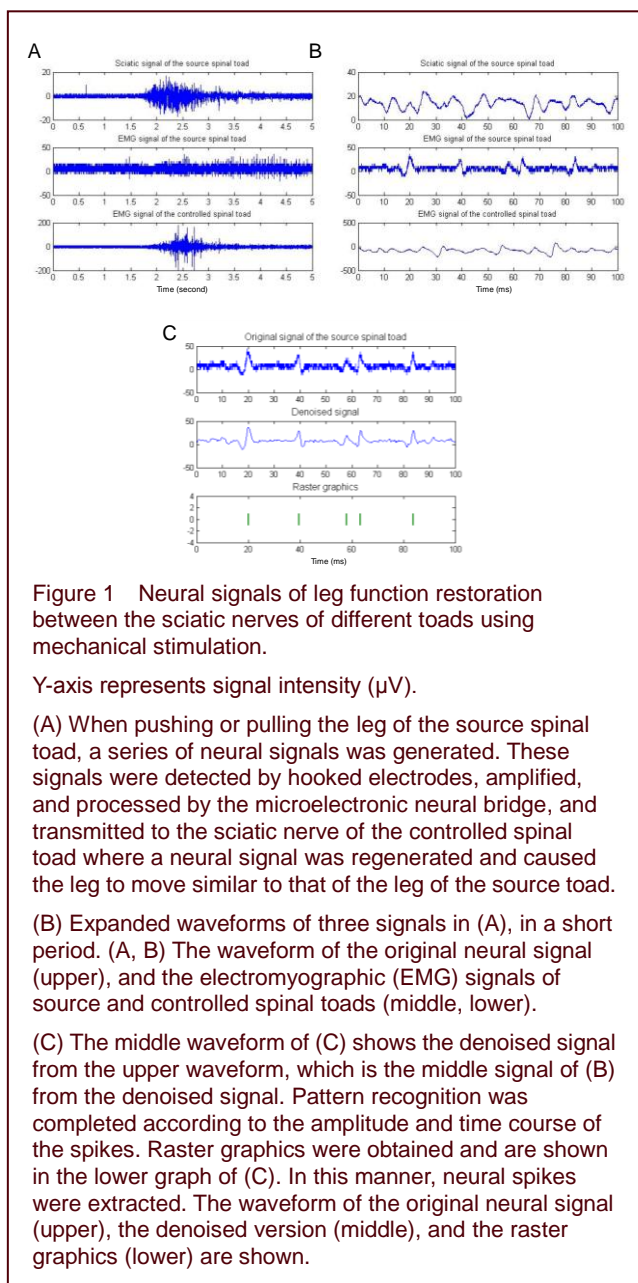Leg function restoration *via* the sciatic nerves of two separated toads was studied. First, mechanical stimulation was introduced so that the signals caused by stimulation, which were used as the control signals of functional electrical stimulation, were guaranteed to be neural signals without artefact.

Two spinal toads were placed on a board and their bellies fixed. In addition to the two pairs of hooked electrodes mentioned above, a third pair of hooked electrodes was hitched on the sciatic nerves of the controlled spinal toad so that the regenerated sciatic signal could be monitored. Two pairs of needle electrodes were inserted into the muscles of the left legs of the two toads and used for electromyographic signal recording. To reduce artefacts, a metal coil was circled between the hooked electrodes and the monitoring electrode on the leg of each toad and grounded. The spinal toads were motionless in the absence of external stimulation. With the application of mechanical stimulation, *i.e.*, pushing and pulling on the leg of the source spinal toad, the source toad moved its leg. This evoked a neural signal, with a series of spikes, in the sciatic nerve of the source spinal toad. The signal was detected by the hooked electrode, amplified and processed by the microelectronic neural bridge, and transmitted to the sciatic nerve of the controlled spinal toad where a neural signal was regenerated, and caused the leg to move similar to that of the leg of the source toad.

In these experiments, corresponding muscle movements of the controlled spinal toad were observed when the left leg of the source spinal toad was pushed and pulled by hand (supplementary Video 1 online). The sciatic neural signal of the source spinal toad and electromyographic signals of the two spinal toads were recorded, as shown in Figure 1.

The coherent function of the signal generated by mechanical stimulation was calculated. The
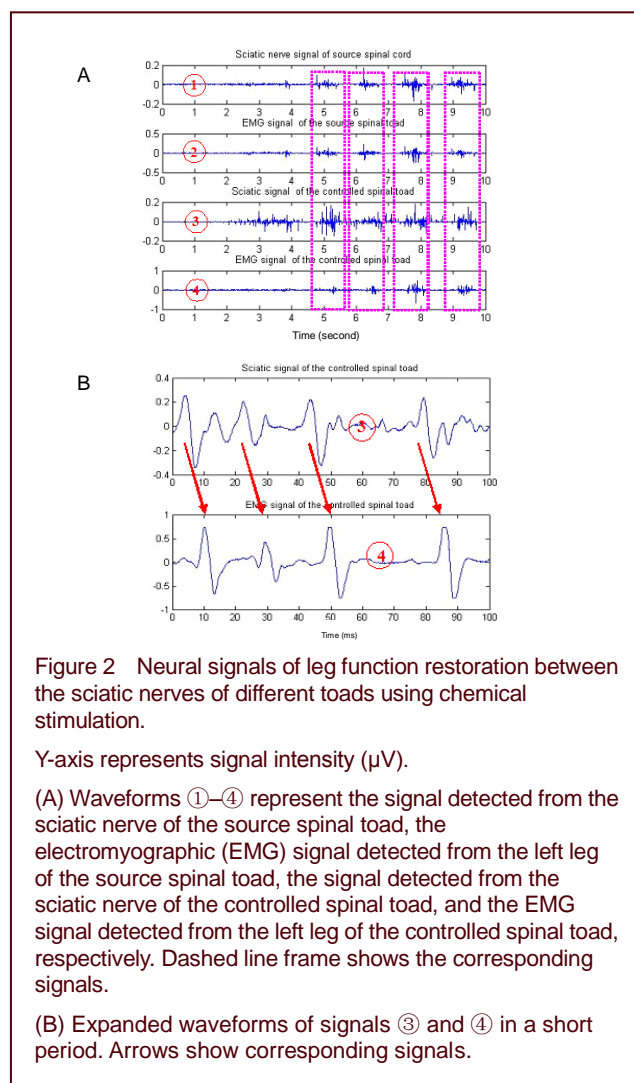
electromyographic signal of the controlled toad was delayed for 0.36 ms in relation to the regenerated sciatic signal, and the coherence function reached a maximum of 0.512 9, whereas the coherence function was a maximum of 0.241 and the delay time was 1.544 ms between the electromyography of the controlled spinal toad and electromyography of the source spinal toad.



Figure 1　Neural signals of leg function restoration between the sciatic nerves of different toads using mechanical stimulation.

Y-axis represents signal intensity (μV).

(A) When pushing or pulling the leg of the source spinal toad, a series of neural signals was generated. These signals were detected by hooked electrodes, amplified, and processed by the microelectronic neural bridge, and transmitted to the sciatic nerve of the controlled spinal toad where a neural signal was regenerated and caused the leg to move similar to that of the leg of the source toad.

(B) Expanded waveforms of three signals in (A), in a short period. (A, B) The waveform of the original neural signal (upper), and the electromyographic (EMG) signals of source and controlled spinal toads (middle, lower).

(C) The middle waveform of (C) shows the denoised signal from the upper waveform, which is the middle signal of (B) from the denoised signal. Pattern recognition was completed according to the amplitude and time course of the spikes. Raster graphics were obtained and are shown in the lower graph of (C). In this manner, neural spikes were extracted. The waveform of the original neural signal (upper), the denoised version (middle), and the raster graphics (lower) are shown.

## Restoration of leg function *via* the sciatic nerves of different toads using chemical stimulation

Subsequently, we applied a drop of 1% sulphuric acid to the toe of the left leg of the source spinal toad to demonstrate the feasibility of neural function restoration using our microelectronic neural bridge. The same experimental set-up as the mechanical stimulation experiments was used (supplementary Video 2 online).

Both the neural signals of the left sciatic nerves and the electromyographic signals of the two left legs of two spinal toads were recorded as shown in Figure 2. Here, waveforms ①–④ represent the signal detected from the sciatic nerve of the source spinal toad, the electromyographic signal detected from the left leg of the source spinal toad, the signal detected from the sciatic nerve of the controlled spinal toad, and the electromyographic signal detected from the left leg of the controlled spinal toad, respectively. To obtain a clearer observation, the expanded waveforms of signals shown in Figure 2A ③ and ④ during a short period are shown in Figure 2B.



Figure 2　Neural signals of leg function restoration between the sciatic nerves of different toads using chemical stimulation.

Y-axis represents signal intensity (μV).

(A) Waveforms ①–④ represent the signal detected from the sciatic nerve of the source spinal toad, the electromyographic (EMG) signal detected from the left leg of the source spinal toad, the signal detected from the sciatic nerve of the controlled spinal toad, and the EMG signal detected from the left leg of the controlled spinal toad, respectively. Dashed line frame shows the corresponding signals.

(B) Expanded waveforms of signals ③ and ④ in a short period. Arrows show corresponding signals.

We calculated the coherent function of signals ① and ③ and signals ③ and ④ in Figure 2. The results show that when the sciatic nerve signal of the controlled spinal toad was delayed for 0.3 ms in relation to the sciatic signal of the source spinal toad, the cross-correlation function reached a maximum of 0.967 7. The electromyographic signal was delayed for 6.5 ms in relation to its regenerated sciatic signal and the cross-correlation

coefficient was 0.840 6.

## DISCUSSION

Mammals such as rats, rabbits, or dogs are usually used as experimental models because the anatomical organisation of their nervous systems are similar to those of humans. However, under most conditions, higher-order vertebrates must be anaesthetised before function restoration experiments can be carried out. Anaesthesia, however, inhibits the excitement of the nervous system[24-25]. There is also a need to study neural signals as indispensable signal sources. This means that mammals may not be the optimal animal model for demonstrating neural signal regeneration and function restoration. In this study we used spinal toads for two reasons: (1) since anesthesia is not required for a toad that is not a higher-class vertebrate, the generation and transmission of neural signals are not inhibited and the generation of neural signals by functional electrical stimulation is not inhibited; (2) as amphibians, toads can maintain biological activity for a long time without control exerted by the brain. Both advantages are useful for experiments investigating neural signal regeneration.

Our goal was to set up a microelectronic neural bridge between two sciatic nerves in different toads and to restore leg function of the controlled spinal toad. To verify the success of the microelectronic neural bridge, we examined the similarity, synchronism or functional coupling of the source signal, the regenerated signal of the sciatic nerve, and electromyographic signals through correlation function analysis. Thus, we analysed the correlation between sciatic signals and electromyographic signals in two experimental strategies with two different stimulations.

The nervous system of an animal is a complex communication network. The aim of our animal experiments was to determine whether a microelectronic module can bridge an injured nerve and achieve function restoration. From the two experimental strategies above, we obtained the following information.

(1) In Figure 2A, waveform ③ represents the regenerated signal in the sciatic nerve of the controlled spinal toad. It is very similar to its source signal, namely waveform ①, the signal in the sciatic nerve of the source toad. The coherence value was 0.967 7 with a delay of 0.3 ms. The results demonstrate that through the microelectronic neural bridge, the signal in the sciatic

nerve of the source toad was successfully transmitted to the sciatic nerve of the controlled toad. Furthermore, the existence of a delay time suggests that the signal in the sciatic nerve of the controlled spinal toad was regenerated, rather than it being artefact from the stimulus, because if it was an artefact there would be no delay between signals.

(2) Figure 2B is one segment captured from Figure 2A and represents an interesting discussion point. By observing and calculating the rough relationship between waveform ③ of the signal from the sciatic nerve and waveform ④ of the electromyographic signal of the controlled toad, it can be seen that the electromyographic signal and its source signal, *i.e.*, the signal from the sciatic nerve, have a definitive relationship as indicated by the arrows. The electromyographic signal was delayed for 6.5 ms in relation to the regenerated sciatic signal, and the cross-correlation coefficient was 0.840 6. This shows that there is causality between the movement and the neural signal in the sciatic nerve.

(3) Figure 2B shows that the electromyographic signal of the controlled toad is indeed a regenerated signal rather than artefact. If it were artefact, in addition to the lack of delay time, it would include all small waves in its source signal.

(4) In Figure 1B, the electromyographic signal of the controlled toad was delayed for 0.36 ms in relation to the regenerated neural signal on the sciatic nerve, and the cross-correlation coefficient was 0.512 9, while the correlation coefficient between the second (electromyography of source spinal toad) and third (electromyography of controlled spinal toad) signals was only 0.241. This suggests that the electromyographic signal is coherent with the neural signal in the sciatic nerve, but the consistency of the actions of the two spinal toads was not as good as expected. This discrepancy may have been caused by unmatched positions of electrodes.

(5) In previous experiments[16] and in our experiments of neural signal regeneration between two separated sciatic nerves with external electrical stimulation, only muscle twitches were observed. When we began our experiments, we hypothesised that several channels of neural signal regeneration would be needed to generate coordinated motion in the controlled spinal toad. In reality, only one channel of the regenerated neural signal was needed to generate a coordinated motion in the controlled spinal toad. This result is significant for neural

function restoration, and the underlying mechanism and reasons why only one channel was needed should be investigated.

We designed two experimental schemas to verify the feasibility of using a microelectronic neural bridge. Leg function restoration between two legs of separate toads was demonstrated. These experiments demonstrated that spinal toads are ideal for experiments of neural function restoration. Recording neural signals was easily achieved, and reliable recordings from the sciatic nerve were made for up to 5 hours. The microelectronics modules we designed can be used successfully for signal regeneration and function restoration under different stimulations.

Future work will focus on the design of implantable microelectronic systems as described above to investigate neural function restoration after spinal cord injury. The ultimate goal is to develop a miniature implantable electronic system that can form a bidirectional signal channel bridge of the spinal cord to substantially improve or restore motility. Successful demonstration of the envisioned approach will pave the way for a useful tool in therapy aimed at function restoration.

## MATERIALS AND METHODS

### Design
A self-controlled study.

### Time and setting
The experiments were performed at Nantong University and Southeast University, China between July 2009 and December 2011.

### Materials
#### Animals
Spinal toads, weighing 45–55 g, were purchased from Shengming Research Animal Farms, Nanjing, China. Toads were kept in indoor round aquariums, with 20 cm depth of well water or aerated tap water over night. Water temperature varied between 12–25°C. A small quantity of pebbles and stones was used for barricades and caves. Three 20 watt fluorescent bulbs were used as the light source, and a water submersible pump was used to add oxygen, and a heater used to heat. All experimental procedures were performed in accordance with *the Guide for the Care and Use of Laboratory Animals*, published by the National Institute of Health.

### Instrumentation
Composition of the microelectronic neural bridge: In principle, a neural bridge is used to provide a multi-channel link across injured nerve fibres to bypass interrupted neural signals[26-27]. The block diagram of our microelectronic neural bridge is shown in Figure 3. It consists of an electrode array, a head stage, a filtering network, a preamplifier, a main amplifier, an functional electrical stimulation driver, and another electrode array. The first electrode array is used to detect the neural signal from the proximal stump of injured nerve fibres. The head stage, the filtering network, and the preamplifier preliminarily amplify the neural signal, but limits interference. After further amplification by the main amplifier, the neural signal is sent to the functional electrical stimulation driver, which supplies a high voltage or a strong current to the second electrode array so that the desired neural signal can be regenerated in the distal nerve stump.



Figure 3   Block diagram of the microelectronic neural bridge.

FES: Functional electrical stimulation.
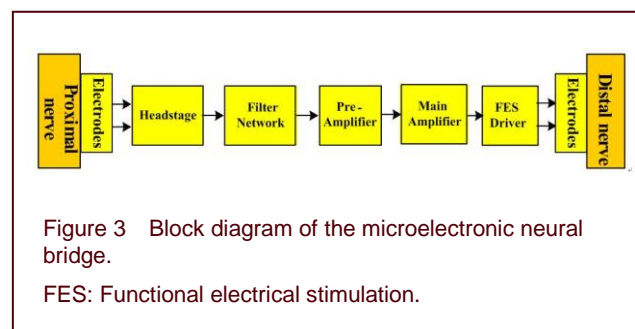
It is challenging to detect a neural signal from a neuron or nerve fibre because the signal is relatively weak and there is considerable noise and interference. Noise for the first stage should be as low as possible. Because the impedance of the signal source, *i.e.* the organism, is high and uncertain, the equivalent current noise is the main consideration. Therefore, a unit-gain buffer is used as the head stage. The filtering network is made up of a low-pass filter that provides attenuation to high-frequency noise and interference before the preamplifier. To reduce the common-mode interference in the lower frequency band, a differential input system with a 128-dB common mode reject ratio was designed. For the purpose of noise reduction, operational amplifiers with an ultra-low noise figure were adopted (Figure 4). The system's 3-dB bandwidth is from 10 Hz to 2 kHz. The output current of the functional electrical stimulation driver is adjustable from 0 to 7.8 mA.

Electrodes: Two types of electrodes, hooked electrodes (Quanshui Experimental Devices Co., Nanjing, China) and self-made needle electrode arrays, were used. The hooked electrodes were made of two parallel metal wires

pressed into the surface layer of a hook-formed plastic plate. To meet the electrochemical requirements and to get "smooth" geometry for the prevention of mechanical trauma and irritation caused by sharp edges, polyimide was used as the substrate and insulation material. Polyimide has been shown to be a non-toxic material suitable for neural implantation[28-30]. The self-made needle electrodes arrays consisted of acupuncture needles covered with Parylene C (Cookson Electronics Specialty Coating Branch, Shanghai, China) except at the tips. The needles with exposed tips were thin and suitable for selective activation of motor nerves.
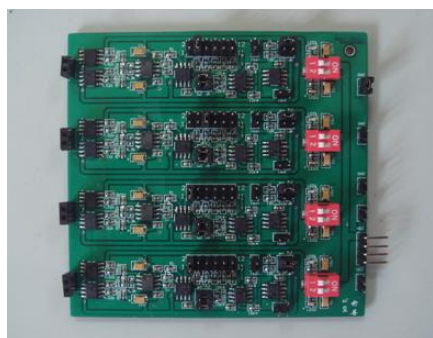


Figure 4    Printed circuit board of the 4-channel microelectronic neural bridge comprised of operational amplifier integrated circuits and discrete devices.

## Methods

### *Establishment of the spinal toad model*

Before experiments, two spinal toads were prepared[31]. The toads were stunned by a blow to the head. A fine scalpel blade was inserted into the spinal canal at the back of the skull severing the brain from the spinal cord. A blunt needle was then inserted through the hole made by the scalpel blade and pushed forward into the skull via the foramen magnum to destroy the brain. A longitudinal incision was made through the skin of the dorsal side of the upper leg, and the sciatic nerve exposed by pulling apart the muscle bundles. The sciatic nerve was freed from surrounding tissue, with care taken to avoid damage to the femoral artery and vein that run alongside the nerve. The sciatic nerve was exposed between the biceps femora and semimembranosus muscles. A small wad of absorbent cotton soaked in Ringer solution was placed over the nerve to prevent drying. The spinal toads recovered from spinal shock within 10 minutes.

The experimental set-up is depicted in Figure 5. The hooked electrodes were in contact with the sciatic nerve of the source spinal toad, which was stimulated by external stimulation. The cable connector was connected to the input of the microelectronic neural bridge.

Simultaneously, the neural signal detected from the sciatic nerve was monitored and recorded using an oscilloscope (Agilent 64200 or Agilent DSO6014A, CA, USA). Another hooked electrode connected with the output of the microelectronic neural bridge contacted the sciatic nerve of the controlled spinal toad. The self-made acupuncture needle electrodes were fixed to the optimal sites for monitoring of electromyographic signals: at the left legs of both spinal toads and under the bellies of the gastrocnemius muscles.
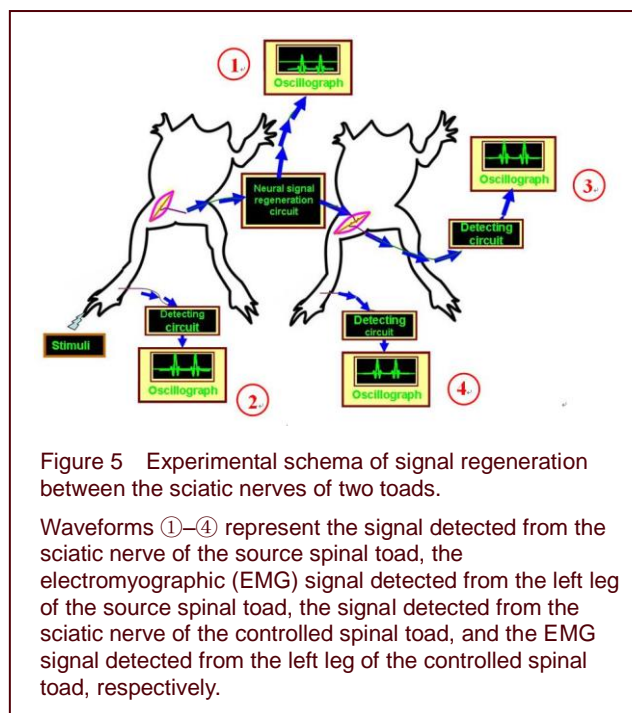


Figure 5    Experimental schema of signal regeneration between the sciatic nerves of two toads.

Waveforms ①–④ represent the signal detected from the sciatic nerve of the source spinal toad, the electromyographic (EMG) signal detected from the left leg of the source spinal toad, the signal detected from the sciatic nerve of the controlled spinal toad, and the EMG signal detected from the left leg of the controlled spinal toad, respectively.

### *Data processing*

The signals acquired by the electrodes were monitored and digitally recorded by the oscilloscope (Agilent 64200 or Agilent DSO6014A, USA). The recorded data were analysed offline using Matlab (MathWorks, MA, USA). Before the analysis of functional coupling between muscular activity and simultaneously recorded oscillatory neural activities of the motor system, denoising and pattern recognition were carried out using wavelet transformation. Wavelet transformation provides a method of decomposing signals both in scale and time. During the last decade, wavelet transformation has been used in different areas of engineering, biology, and medicine[32-34]. Because of its superiority in analysing transient and non-stationary signals, it has become a powerful tool and extension of Fourier analysis. Most biomedical signals are not stationary and have highly complex time-frequency characteristics. The wavelet method can provide good time resolution at high frequency and good scale resolution at low frequency[35]. The wavelet function used was DB3 wavelets[36]. After

wavelet transformation, pattern recognition was completed according to the amplitude and time course of the spikes. Finally, raster graphics were obtained, and neural spikes extracted.

The analysis of functional coupling between muscular activities, while simultaneously recording oscillatory neural activity at different levels of the motor system, has provided significant insights into the neural control of movement[37-39]. One widely used method of estimating the similarity, synchronism, or functional coupling between two oscillatory signals is the coherence function $\rho_{xy}$, defined by[40]

$$\rho_{xy}^2 = \frac{\left(\sum_{n=-\infty}^{+\infty} x(n)\,y(n+m)\right)^2}{\sum_{n=-\infty}^{+\infty} x^2(n) \sum_{n=-\infty}^{+\infty} y^2(n)}$$

where $x(n)$ and $y(n)$ are discrete signals; m is the number of shift points and delay time; $m > 0$ means that sequence $y(n)$ shifts left, while $m < 0$ means that sequence $y(n)$ shifts right.

The coherence function is a normalized correlation function, and measures the relative linearity and delay time between two signals. The value of coherence has no relationship with the magnitude of oscillatory signals, and is convenient for comparing the degree of relativity. It can not only express phase coherence, but can also express phase-shift (or delayed) coherence. The coherence value indicates the strength of the coupling in the time domain. It is mathematically bounded between 1 and +1, where 1 indicates a perfect linear relationship and 0 indicates no linear relationship.

The coherent function was calculated not only between the sciatic neural signals of source spinal toad and controlled spinal toad, but also between electromyographic and sciatic neural signals. The phase differences provided an estimation of the temporal delays between those signals.

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