Original Article
Comparison of two electrophysiological methods for the assessment of progress in a rat model of nerve repair

Dong Han[1,2,3], Jiuzhou Lu[1,2,3], Lei Xu[1,2,3], Jianguang Xu[1,2,3]

[1]Key Laboratory of Hand Reconstruction, Ministry of Health, Shanghai 200032, China; [2]Shanghai Key Laboratory of Peripheral Nerve and Microsurgery, Shanghai 200032, China; [3]Department of Hand Surgery, Huashan Hospital, Fudan University, Shanghai 200040, China

Abstract: There are 2 critical steps in neural regeneration: nerve fibres successfully crossing the suture and restoration of neuromuscular transmission. For the second step, the compound muscle action potential (CMAP) is the standard electrophysiological technique used to assess regeneration, but it is difficult to detect changes in the CMAP during early regeneration after nerve repair. There is a need for better, noninvasive quantitative electrophysiological techniques to assess regeneration in an earlier stage after nerve repair. In this study, we utilized 2 measures, CMAP and single-fibre electromyography (SFEMG), in a rat model of nerve repair. The model was generated by separating the sciatic nerve of the rat hindlimb from the tibial nerve in Sprague-Dawley rats. CMAP and SFEMG were measured in each rat at 1, 2, 3, 4, and 6 weeks after the operation. The muscle weight was measured and both the general structure of the muscle and the changes in muscle atrophy were examined using haematoxylin and eosin staining protocols. The nerve electrophysiological data could be detected at 2 weeks after surgery initially and more data could be collected with passing time. During the period ranging from 2 to 4 weeks after surgery, parameters of SFEMG recordings changed significantly while the CMAP amplitude did not increase until 6 weeks after surgery. While the fibre density (FD) at 2 weeks after surgery was 0.27 ± 0.31, there was a significant increase at 3 weeks relative to 2 weeks (P < 0.01), and the FD increased further at 4 weeks (P < 0.01). The action potential mean consecutive difference (MCD) was significantly higher (60.50 ± 3.53 µs) in the second week relative to the third week (41.12 ± 5.08 µs) after the operation. The results indicated that SFEMG was more sensitive than CMAP amplitudes in detecting neuromuscular transmission after nerve repair. The findings of nerve electrophysiological experiments were consistent with the observed degree of muscle recovery. The SFEMG can be used to detect the very early reinnervation of the muscle more sensitively than CMAP. The ratio of affected muscle weight to unaffected muscle weight was decreased at 2 weeks after surgery (59.01%), continued to decrease significantly at 3 weeks (51.24%), and was restored at 6 weeks. A combination of SFEMG and CMAP can show the dynamic progression of the muscle reinnervation process.

Keywords: Peripheral nerve injury, single-fiber electromyography, regeneration, nerve repair

Introduction

Peripheral nerve injury is frequently caused by various traumatic injuries. The results following nerve repairs are influenced by many parameters. These include the nature, location, and extent of the injury; the level and timing of the repair, surgical technique, and patient factors [1]. Regeneration of the damaged peripheral nerve depends on whether the repaired nerve could pass through the nerve suture site and whether it can carry neuromuscular transmission functions successfully.

Traditional methods to judge the extent of the earliest regenerated nerve neuromuscular transmission include manual testing of muscle strength using the UK Medical Research Council (MRC) score [2], dynamometry [3] to demonstrate severe weakness, electrophysiological tests to explore the function of peripheral nerves and muscles, and muscle biopsy. Compound muscle action potential (CMAP) represents the summation of the action potentials of all excited muscle fibres that respond to the nerve stimulation. Conventional nerve conduction studies (NCS) with measurement of CMAP

amplitudes are most commonly used as indicators for neural regeneration [4]. These and other specialized techniques such as direct muscle stimulation or axonal excitability testing may reveal nerve or muscle dysfunction with a high degree of specificity. However, these techniques do not allow diagnosis of small intraepidermal nerve fibre pathology in the early stage of nerve repair, due to the minimal amplitude of CMAP caused by the diversity of nerve conduction.

Single-fibre electromyography (SFEMG) is the most sensitive technique for detecting abnormalities of neuromuscular transmission. SFEMG investigations of peripheral nerves and muscles offer several advantages. The single-fibre needle electrode (SFE) has a small recording surface (25 μm in diameter) that is exposed at a port on the side of the electrode (3 mm from the tip), which permits identification of a single muscle fibre action potential, and enables measurement of jitter and fibre density. SFEMG is much more sensitive than traditional electromyography and the most sensitive method for detecting neuromuscular junction disorders [5, 6]. SFEMG can also be used to detect the process of axonal regeneration and reinnervation after nerve repair as an effective indicator of determining the neurological recovery function. Jitter reflects neuromuscular function, which can be expressed as action potential mean consecutive difference (MCD); increasing MCD represents neurological defects. Fibre density (FD) reflects the distribution of muscle fibres per motor unit. Collateral sprouting can rebuild a motor unit and increase the quantity and density of muscle fibres. In this study, we used the FD to observe the number of nerve fibres, and action potential MCD to assess neuromuscular function. Neuromuscular junction function is mainly indicated by MCD. The aim of the present study was to investigate whether jitter of SFEMG analysis can be used for detection of reinnervation of neuromuscular transmission by regenerated nerves.

Materials and methods

Animals

A total of 30 adult female Sprague-Dawley rats of clean grade II, weighing 150-200 g, were provided by the animal centre of Fudan University. Experimental protocols were approved by the Animal Ethics Committee of Fudan University.

Generation of animal models

Thirty rats were intraperitoneally injected with 10% chloral hydrate solution (0.3 ml/kg body weight) and fixed on the operation bench. The nerve repair rat models were established as follows: the right hindlimb sciatic nerve was exposed and cut off at the level of its separation from the tibial nerve, peroneal nerve, and sural nerve. The common peroneal nerve was resected at the point it enters the muscle. The proximal sciatic nerve and distal tibial nerve were sutured at × 10 magnification. The left hindlimbs were used as controls.

CMAP and SFEMG measurements

CMAP and SFEMG were recorded in each rat at 1, 2, 3, 4, and 6 weeks after the operation using Alpine BioMed ApS (Denmark). Rats were intraperitoneally anaesthetized using 10% chloral hydrate (0.3 ml/kg) and fixed on the operation bench. For CMAP, the recording electrode was placed on the muscle belly. The electrical stimulation parameters were as follows: stimulus frequency 1 Hz, intensity 4 mA, discharge 100 times. 6 single-fibre images were collected in different locations of the muscle. For SFEMG, stimulating needle electrodes were inserted into the entrance points of the muscular nerve and single-fibre recording needle electrodes were inserted into the triceps surae. The inserted electrodes were continuously moving to find the appropriate single-fibre action potentials (the peak-peak amplitude > 200 μV; the time of positive peak-negative peak < 300 μs; stable continuous waveform) [7]. The electrical stimulation parameters were: stimulus frequency 1 Hz, intensity 4 mA, discharge 100 times. 6 SFEMG images were collected in different locations of the gastrocnemius muscle and automatically stored on computer to analyze the action potential MCD and muscle FD. Single-fibre EMG techniques included 2 parameters: jitter and FD. Jitter is also known as the MCD and neuromuscular dysfunction may prolong MCD. FD refers to the number of single-fibre action potentials in the range of 300 μm electrodes, and increases in FD indicate reinnervation.
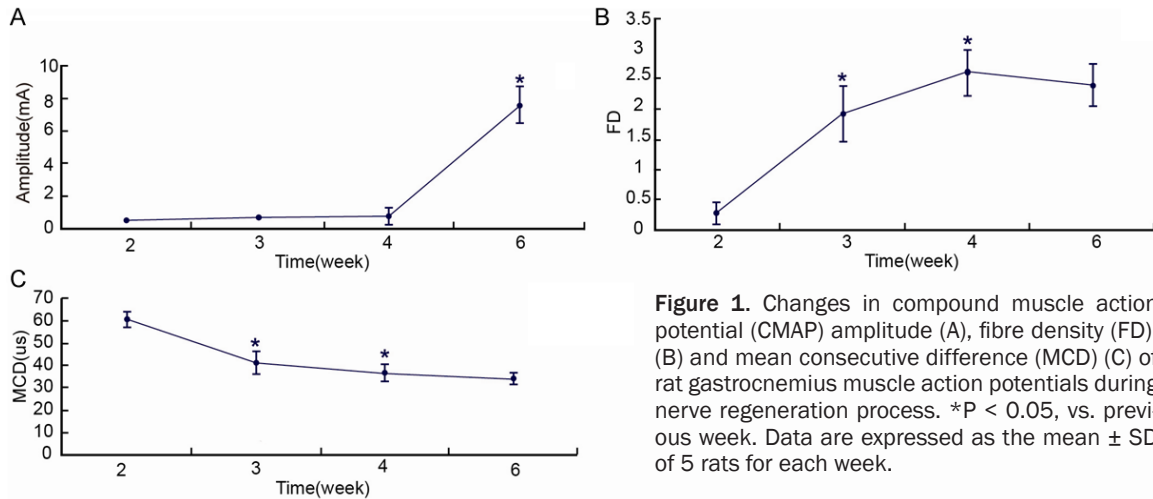
Figure 1. Changes in compound muscle action potential (CMAP) amplitude (A), fibre density (FD), (B) and mean consecutive difference (MCD) (C) of rat gastrocnemius muscle action potentials during nerve regeneration process. *P < 0.05, vs. previous week. Data are expressed as the mean ± SD of 5 rats for each week.

Muscle weight and pathological observation

The entire bilateral triceps surae was immediately excised from its proximal end just below the knee to its distal end at the gastrocnemius tendon, and its mass was obtained. A series of 5-μm-thick sections of formalin-fixed muscle were embedded in paraffin and used for histopathological staining. The general structure of the muscle and the changes in muscle atrophy were examined using haematoxylin and eosin staining protocols. The slides were observed with an optical microscope at a low-power field magnification of × 10 using 2 slices from each of 5 rats at each time point. The images were photographed using a JVC1381 color photography camera (Nikon, Tokyo, Japan).

Statistical analysis

Measurement data were expressed as mean ± SD, and analyzed using SPSS 19.0 software (SPSS, Chicago, IL, USA). For all statistical tests, significance was determined at P < 0.05. In order to effectively capture all the data from multiple recordings over time in the comparison between groups, Mann-Whitney U tests were performed to analyze the amplitude differences of CMAP among different weeks. In order to utilize all the data obtained, differences between MCD were initially assessed via repeated 2-way ANOVA, in which the independent variable was group and the dependent variable was the individual electrophysiological measures.

Results

The nerve neural stem action potential (NAP) data could be detected at 2 weeks after surgery and more data could be collected with the passage of time. Representative data from each recording technique are shown in **Figure 2**. There was no apparent increase in CMAP amplitude until 6 weeks after surgery (P < 0.01) (**Figure 1A**). While the FD at 2 weeks was 0.27 ± 0.31, there was a significant increase at 3 weeks relative to 2 weeks (P < 0.01), and the FD continued to increase at 4 weeks (P < 0.01) (**Figure 1B**). The mean MCD was significantly higher (60.50 ± 3.53 μs) in the second week relative to the third week (41.12 ± 5.08) μs after surgery (P < 0.01). The mean MCD continued to decrease during the following week. There were also significant differences between the third week and either the fourth week or the sixth week (P < 0.05). The mean MCD did not change significantly from 4 to 6 weeks (**Figure 1C**). The results indicated that SFEMG was more sensitive than CMAP amplitudes in detecting neuromuscular transmission after nerve repair.

Additionally, the entire bilateral triceps surae was excised from its proximal end just below the knee to its distal end at the gastrocnemius tendon, and its mass was obtained. The ratio of right muscle weight/left muscle weight (i.e. affected side/unaffected side) was calculated to evaluate muscle conditions. The weight ratio was decreased 2 weeks after surgery (59.01%) and was significantly further decreased at 3 weeks (51.24%), but was restored at 6 weeks
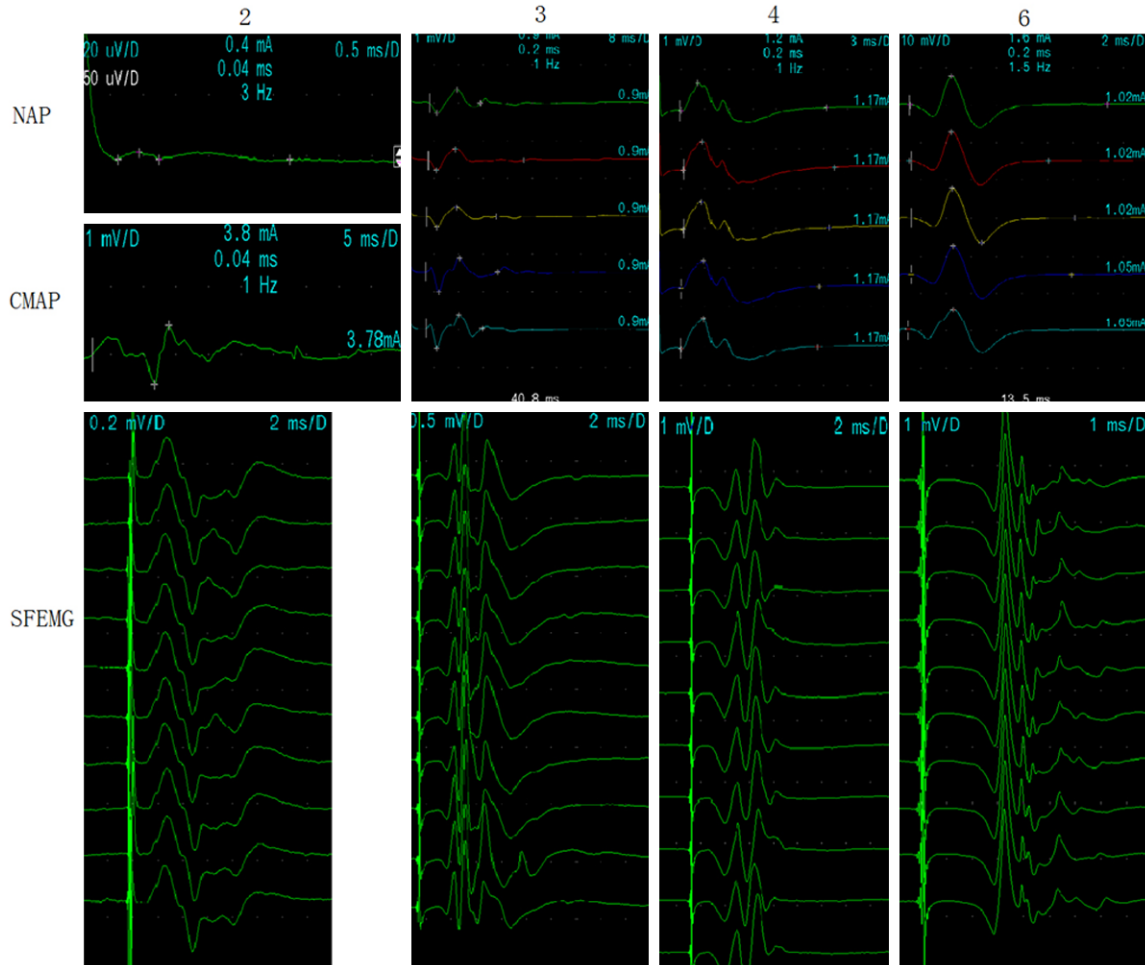
Figure 2. Representative samples showing NAP, CMAP, and SFEMG results at 2, 3, 4, and 6 weeks after surgery. NAP, neural stem action potential; CMAP, compound muscle action potential; SFEMG, single-fibre electromyography.

Table 1. Muscle weight in rat model of nerve repair at each week (*p* value vs. previous week)

Week		1	2	3	4	6
Muscle weight	Affected side/Unaffected side	69.21%	59.01%	51.24%	53.13%	68.31%
P		-	0.002	0.037	0.683	0.002

(68.31%) (**Table 1**). The general structure of the muscle and the changes in muscle atrophy were examined (**Figure 3**). Muscle atrophy was observed during the first week. This increased in the following weeks, but was restored at 6 weeks. Our nerve electrophysiological findings are consistent with the degree of muscle recovery directly observed.

Discussion

The peripheral nerve system (PNS) is composed of the cranial nerves, which project from the brain and passes through the foramina in the skull; and the spinal nerves, which project from the spinal cord and pass through the intervertebal foramina of the vertebrae [8]. The PNS consists of motor and sensory neurons that are the largest and most spatially complex in the body. Peripheral nerve injuries are more frequent and may be accompanied by neurological deficits. In contrast to the central nervous system, the PNS is capable of regenerating injured axons [9]. Regeneration of the damaged peripheral nerve depends on the microsurgical procedure performed. The results following
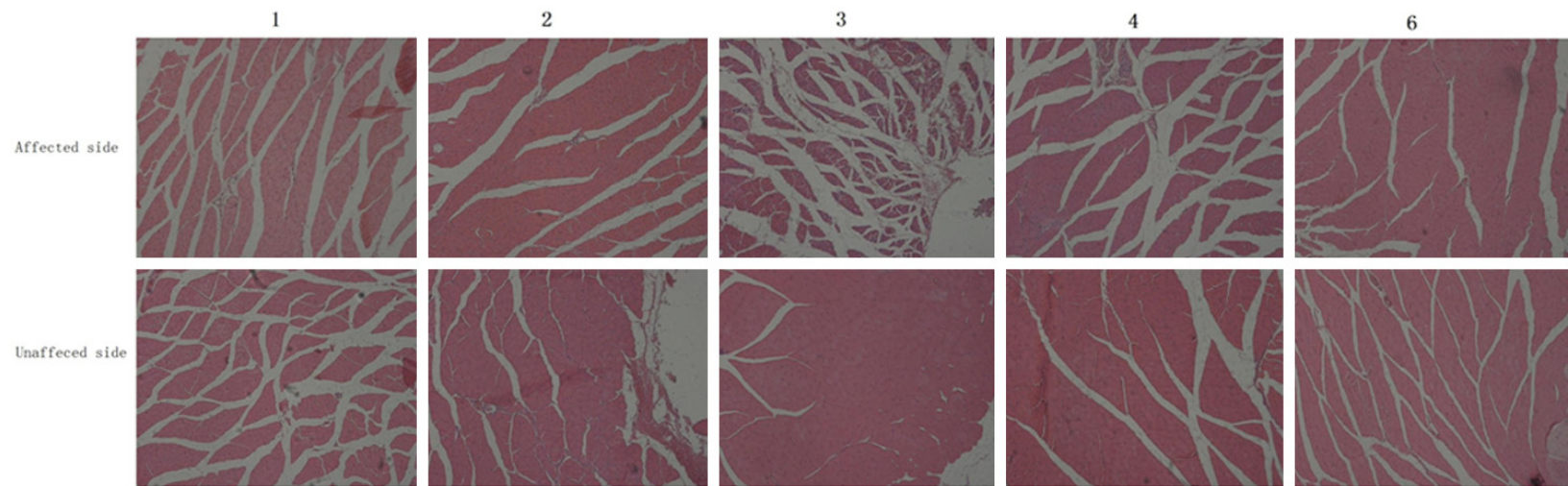
Figure 3. The changes in muscle atrophy were examined using haematoxylin and eosin staining protocols on tissue isolated at 1, 2, 3, 4, and 6 weeks after surgery. The affected hindlimbs and the unaffected hindlimbs are displayed in the figure as labeled.

nerve repairs are diagnosed by physical examination and electrophysiological methods. There are many indices for evaluating nerve regeneration and we selected 2 indicators for both pathological and electrophysiological evaluation. CMAP amplitudes are most commonly used as indicators for neural regeneration. Investigations of the CMAP amplitudes of peripheral nerves and muscles have several limitations. CMAP does not allow diagnosis of small intraepidermal nerve fibre pathology in the early stage of nerve repair, due to the minimal CMAP amplitude caused by diversity of nerve conduction. Additionally, it is difficult to decide whether any observed failure is in nerve regeneration or due to muscle inactivation. SFEMG is a sensitive indicator of neuromuscular junction function that can reflect the electrical activity of different muscle fibres and their motor end plates within a single motor unit. As such, it is the most sensitive technique for detecting abnormalities of neuromuscular transmission. SFEMG is a selective EMG recording technique that allows identification of action potentials (APs) from individual muscle fibres. The selectivity of the technique results from the small recording surface (25 μm in diameter), which is exposed at a port on the side of the electrode, which is 3 mm from the tip [10]. The measurement parameters include jitter and fibre density, which are indicators of axonal regeneration activity [11]. Jitter reflects neuromuscular function, which can be expressed as action potential MCD; increasing MCD represents neurological defects. FD reflects the distribution of muscle fibres within a motor unit. Collateral sprouting can rebuild a motor unit, and increase the quantity and density of muscle fibres. The normal MCD value varies from 10 to 50 μs among different muscles [12]. In this study, we used the FD to observe the number of nerve fibres, and action potential MCD to assess neuromuscular function. Neuromuscular junction function is mainly indicated by MCD.

Overall, our results support the notion that SFEMG is more sensitive than CMAP for the detection of neuromuscular transmission reinnervation in rat models of nerve repair. Of the 2 electrophysiological biomarkers, both CMAP and SFEMG could reveal significant differences in the progress of nerve repair. However, SFEMG could detect significant differences in the third week, while CMAP could not detect significant differences until the sixth week. In conclusion, the results of this study confirmed the feasibility of jitter analysis with SFEMG and the usefulness of this technique for the detection of neuromuscular transmission. However, this study included only a small number of rats and did not include human trials. The sensitivity and specificity of jitter analysis with SFEMG for the detection of neuromuscular transmission thus needs further investigation.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jianguang Xu, Department of Hand Surgery, Huashan Hospital, Fudan University, No. 12 Middle Wulumuqi Road, Shanghai 200040, China. Tel: +86-21-52888210; Fax: +86-21-52888210; E-mail: xujianguangshhb@126.com

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