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Original Article

Signature Analysis of EMG by Wavelet Packet after TNFR Surgery in Rat Model

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Abstract

Background and objectives: The development of a multifunctional bionic prosthetic system to meet the daily activity needs of amputees has become a research hotspot in the field of international neuroengineering and rehabilitation technology. In order to reconstruct the muscle power supply related to the physiological activities of the lost limb, a method of targeted neural function replacement (TNFR) based on wavelet packet decomposition was proposed.

Methods: The TNFR rat model was established, and the necessary rehabilitation methods were used to restore the movement of rats as much as possible, in order to better collect the electromyography signals in the sham operation group, denervation group, TNFR group, 2-week delayed TNFR group, and 4-week delayed TNFR group. After signal acquisition, wavelet packet transformation was used in the analysis.

Results: Compared to the first week, the average rectified values for the right biceps in the TNFR model group, and 2-week and 4-week delayed surgery groups significantly increased in the fourth week ($p < 0.05$). There was a significant difference between the fourth week TNFR group and 4-week delayed group ($p < 0.05$).

Conclusions: The present results revealed that the neural function reconstruction of the early TNFR operation is better, and that the average rectified values for the target muscle obtained after the operation were enhanced. These preliminary research results obtained from rats can be used as an exploratory basis, in order to provide reliable experimental data for clinical treatment and post-amputation research, which may be helpful in improving the quality of life of amputee patients and their control of the prosthesis.

Keywords: Targeted neural function replacement; Wavelet packet; EMG signal; End-to-end coaptation.

Abbreviations: ARV, average rectified value; EMG, electromyography; TNFR, targeted neural function replacement.

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Introduction

According to the announcement issued by the Leading Group of the Second National Disability Sampling Survey and the National Bureau of Statistics, among the 82.96 million disabled people in China, 24.12 million people are physically disabled, accounting for 29.08% of the total number of disabled persons. These disabled patients often have neural injury, and injured patients present with a series of physiological changes, such as Waller's degeneration, denervated muscle atrophy, and irreversible function damage. Therefore, certain methods need to be used to reconstruct the nerve function, reduce the atrophy of the denervated muscle, and promote its function recovery.[1]

Targeted neural function replacement (TNFR) is a method of

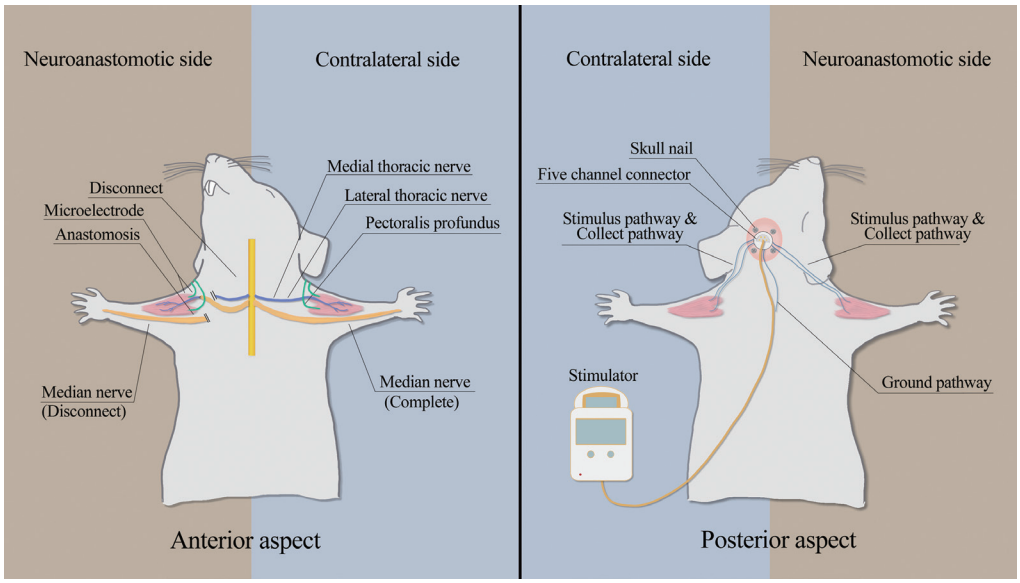

Fig. 1. TNFR EMG acquisition mode diagram. The skull attachment used a 5-channel external connector electrode with two lead wires attached to each channel. EMG, electromyography; TNFR, targeted neural function replacement.

end-to-end anastomosis between the target nerve and primary innervated nerve of the target muscle, and the target nerve is used to replace the original nerve function to complete the target nerve reinnervation of the target muscle.[2] Its advantage is that the original neural network structure of the targeted muscle is used for reinnervation, which can not only slow down the atrophy speed of the targeted muscle, but also recover parts of the electromyography (EMG) signal. These signals can be more precisely and quickly analyzed using wavelet packet transformation, making these a clear and effective signal source,[3] and allowing for a more accurate amputation and control of the prosthesis. In addition, this can reduce the physical and mental burdens of amputees, effectively improve their quality of life, and promote social activities.

The present study aims to demonstrate the effect of surgery on the enhanced signal source using invasive rat intramuscular EMG acquisition, which is more accurate when compared to human non-invasive surface EMG acquisition.

Materials and methods

Animals and groupings

Thirty SPF adult male Sprague-Dawley rats (7–8 weeks old, bodyweight: 220–250 g) were provided by the Animal Experimental Center of Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences (Experimental animal license no.: syxk [Yue] 2012–0119). These rats were randomly divided into three groups: sham operation group, denervation group, and TNFR group. According to the timing of meridian anastomosis, the TNFR group was further divided into the following groups, with six rats in each group: TNFR group, 2-week delayed TNFR group, and 4-week delayed TNFR group.[4] The present experiment was carried out according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (see Supplementary File 1), and approved by the Animal Ethics Committee of Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences.

Preparation of the joint between the electromyography electrode and skull

The experimental electrode was an electrode with a Teflon coating on the outer layer, and the skull joint had a 5-channel external connector electrode with two electrode wires connected to each channel. The ground wire of the reference electrode was placed under the skin of the rat.[4] First, a section of the electrode wire was cut, and the coating on the end was removed. Second, the electrode wire was welded to the skull joint. Finally, the left and right channels were marked. The above steps were repeated to complete the connection for the remaining four channels.

Preoperative preparation of rats

Before the experiment, all experimental equipments were sterilized at a stable high temperature, and rats were fasted for 12 hours, and were forbidden to drink for six hours. During the experiment, these rats were weighed and recorded. Surgical anesthesia was induced by gas anesthesia with 3% isoflurane for approximately 3–5 minutes, and the concentration was maintained at 2% until the end of the operation. After anesthesia, these rats were placed on the operating table, and the limbs were fixed to fully expose the operation area. Then, the surgical site was depilated using a hand-held electric shaver to remove the fur from the surface of the surgical area. Afterwards, the operation area was disinfected using povidone-iodine and deionized using 75% alcohol, and the residual liquid on the surface of the operation area was wiped using sterile gauze. The pattern diagram is presented in Figure 1.

Experimental grouping

For the present experiment, the right upper limb median nerve was selected as the target nerve for the five groups of rats. Furthermore, the musculocutaneous nerve was selected as the target nerve, and the left upper limb was selected as the normal side.

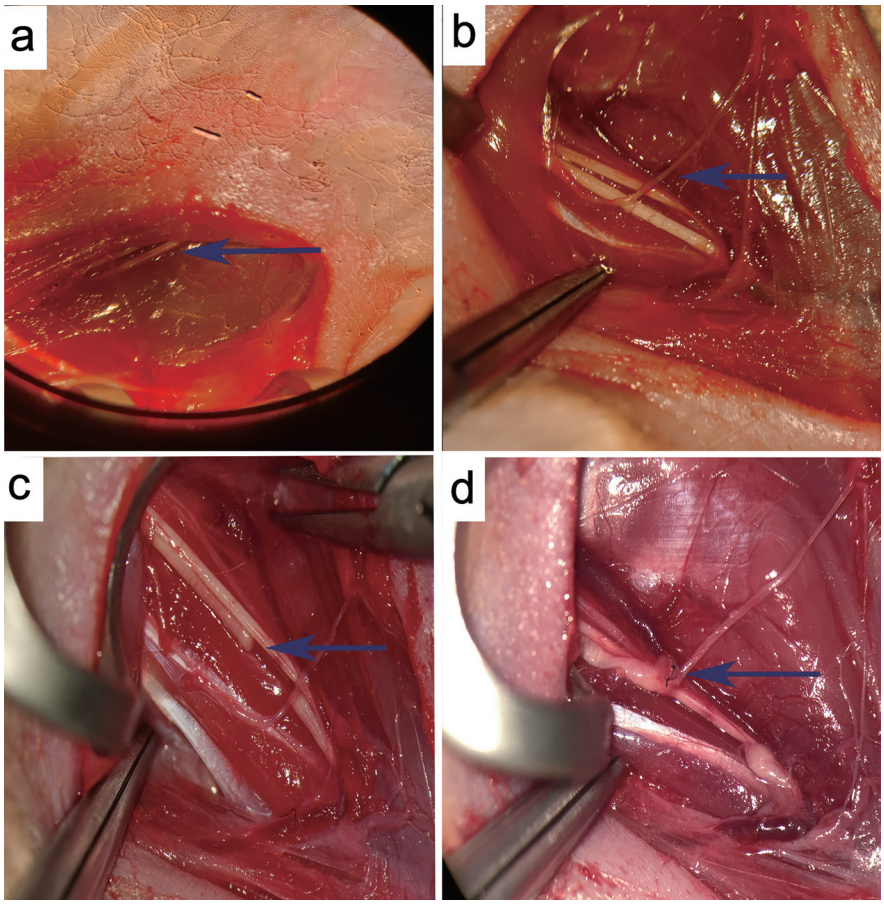

Fig. 2. The combined surgery procedure for the TNFR group. (a) During the end-to-end neurological surgery, the median nerve was exposed (blue arrow); (b) The right cutaneous nerve of the upper arm was protected (blue arrow); (c) The median nerve was cut off (blue arrow); (d) The median nerve and musculocutaneous nerve near the muscle entrance (end-to-end anastomosis) was aligned (blue arrow). TNFR, targeted neural function replacement.

Merely electrode implantation was performed, and there was no muscle separation or nerve exposure. The present method was based on the "nerve end-to-end anastomosis model", as presented below.[4–8] The treatment method used for each group of rats is, as follows[9–10]: the skin, superficial fascia (subcutaneous tissue) and deep fascia were separated layer by layer at the place where the acromion of rats was perpendicular to the midline of the elbow, the pectoralis major and minor muscles were bluntly separated along the muscle fibers, and the brachial plexus was directly exposed. (1) Sham operation group: After anesthesia, merely the median nerve, musculocutaneous nerve and biceps brachii were isolated and exposed, without performing other treatments, and these were sutured layer by layer. (2) Denervation group: After anesthesia, the median nerve, musculocutaneous nerve and biceps brachii were isolated and exposed, the median nerve and musculocutaneous nerve were ligated and severed using a No. 8 suture needle, and the incision area was strictly sutured layer by layer. (3) TNFR group: After anesthesia, the musculocutaneous nerve and median nerve were separated, the musculocutaneous nerve and median nerve were cut off using microsurgical scissors, the nerve was anastomosed (the anastomosis site was located as close as possible to the muscle entry point), and the incision area was strictly sutured layer by layer. (4) TNFR delayed groups (2-week and 4-week delayed groups): After anesthesia, the proximal end of the musculocutaneous nerve and distal end of the median nerve were respectively ligated using a No. 8 suture needle, and these were cut off using microsurgical scissors. Then, the incision area was strictly sutured layer by layer. After 2 and 4 weeks of recovery, the nerve was repaired in the same manner as that in the TNFR group. Then, after the nerve was anastomosed, the electrodes were implanted. Next, a transverse incision was made from the biceps brachii muscle to the elbow joint of the rat, in order to bluntly expose the biceps brachii. Then, a longitudinal incision was made on the scalp of the rat, and the electrode wires of the skull joint were threaded from the subcutaneous sides to the biceps brachii. Afterwards, the biceps brachii was separated and implanted with an electrode wire, and the coating was peeled off at the end of the electrode wire. After determining the position of the electrode wire, the electrode wire was fixed in the muscle.

After the electrodes were implanted, the skull joint was fixed. Then, the rat was placed in the prone position on the operating table, the head was fixed, the scalp was removed, and the fascia was exposed from the perforation to the surface of the skull. Afterwards, six holes were drilled near the lambdoid suture of the skull, skull nails were implanted, and the skull joint was fixed (female connector) into the space by enclosing it with six skull nails. After placing the skull joint, the wound of the skull was cleaned, and the skull joint was fixed on the surface of the skull using ultraviolet curing glue to complete the operation (Fig. 2).

Rat EMG signal acquisition

EMG signals were collected three times a week, from the 1st to the 4th week after surgery. In order to simulate the process of functional recovery of nerve-impaired people, a rat runner treadmill was made, with a diameter of 0.40 m and a circumference of 1.26 m. The speed of the treadmill can reach 30 r/min (0.63 m/s). A reference treadmill training was prepared for these rats. The research reports for the rehabilitation of peripheral nerve injury are presented below.[5,7,11] When the rats ran on the treadmill, the EMG signal was collected using the electrode wire in the biceps brachii muscle, and the output was sent to a self-made EMG signal acquisition module. The self-made EMG signal acquisition module parameters were, as follows: signal amplification factor 1,000, sampling rate 1 kHz, 50 Hz notch (off), sampling interval 0.1 ms, bandwidth 1–400 Hz, and noise floor 1.32 uV. Before collecting these, the speed of the treadmill was adjusted to 3–6 r/min (0.06–0.12 m/s), and the rats were allowed to perform adaptive training for three minutes. After the start of the collection, the rat was induced to run for 30 seconds and rest for 30 seconds. This process was repeated for three times to complete the collection of EMG signals. Then, the collected raw data were imported into MATLAB R2021a for analysis.

Analysis of intramuscular myoelectric signals

The signal recording included EMG signals during the contractions of re-innervated muscle activity and signals during muscle activity, such as interference, noise and motion artifacts. In order to accurately evaluate the function of the biceps and extract clear signals, it was necessary to overcome the problems of poor frequency resolution in other high-frequency bands and poor time resolution in low-frequency bands. Hence, wavelet packet decomposition and reconstruction methods were adopted. Wavelet packet analysis is an integral transform that decomposes signals in the frequency band.[12] By changing the time scale, the local details of the signal can be enlarged and reduced. Wavelet analysis has the characteristics of realizing the multi-resolution transformation of a signal. The algorithm for the wavelet packet decomposition and reconstruction is, as follows:

$$WT(a,\tau)=\frac{1}{\sqrt{a}}\int_{-\infty}^{\infty} f(t)*\Psi(\frac{t-\tau}{a})dt \quad (1)$$

$$\phi(t)=\sqrt{2}\sum_{k} h_{ok}\phi(2t-k) \quad (2)$$

$$\psi(t)=\sqrt{2}\sum_{k} h_{1k}\phi(2t-k) \quad (3)$$

Due to the N = 2 in Daubechies wavelets, the structure of the db2 wavelet was simple and similar to the EMG signal.[13] Therefore, the db2 wavelet basis function was used to decompose the EMG signal collected during the TNFR operation, with three layers of wavelet packets. S1–S8 represent the eight wavelet packet coefficients, respectively, and the corresponding frequency band range of each node was decomposed by wavelet packet.

The average rectified value (ARV) was used as the characteristic indicator for the decomposed and reconstructed EMG signal, in order to measure the activity level of the biceps.

$$X_{arv}=\frac{1}{T}\int_{0}^{t}|x(t)|dx \quad (4)$$

Statistical analysis

Data analysis was performed using the SPSS 21.0 statistical software. The mean of the measurement data ± standard deviation ($\bar{x}$ ± s) was used for comparisons between and within groups performed through a completely random t-test, and $p < 0.05$ was considered statistically significant.

Results

EMG signal decomposition and reconstruction

Through the running training, the five groups of rats completed the running action. With the implanted intramuscular electrodes, the EMG signal was stably recorded from rats while running, and the wavelet packets were used for the decomposition and reconstruction. Figure 3a–d present the EMG signals generated by the biceps of rats in the five groups during the first and fourth weeks of running by decomposition and reconstruction. The red and blue signals in the upper part of the figure represent the EMG signals generated by the right (TNFR side) and left (normal side) of the biceps brachii in the first week after surgery. The red and blue signals in the lower part represent the EMG signals in the fourth week after surgery. These EMG signals were generated by the left and right biceps.

In comparing Figure 3b–c, the TNFR experimental side (red) was able to collect stable EMG signals in the first week. When compared to the denervation group, the signal amplitude in the fourth week was significantly stronger, when compared to that in the denervation group. The EMG signal on the experimental side of the TNFR significantly increased.

Figure 3d–e illustrate the changes in EMG signals in the 2-week delayed TNFR group in the 1st week and 4th week, respectively. In the 4th week, on the experimental side in the delayed repair group at the 2nd and 4th week of repair, the amplitude of the EMG signal significantly increased, but the amplitude remained weaker than that in the sham operation group.

Analysis of active myoelectric signals

The EMG signal itself was one of the wave types. The greater the ARV, the greater the EMG change.[14] Therefore, the ARVs of the EMG signal can be used to reflect the changes in muscle function. The results are presented in Table 1.

Compared to the first week, the ARVs for the right biceps in the TNFR model group, and 2-week and 4-week delayed TNFR groups significantly increased at the fourth week ($p < 0.05$). The ARVs for the right biceps brachii in the normal sham operation group and denervation group approximately remained constant ($p > 0.05$). There was a significant difference between the fourth-week TNFR group and 4-week delayed group ($p < 0.05$), indicating that early TNFR surgery may provide more myoelectric signal sources.

Discussion

The present results revealed that early TNFR surgery has a significant enhancement effect on the acquisition of EMG signals in rats, and that the enhancement effect on EMG acquisition can be

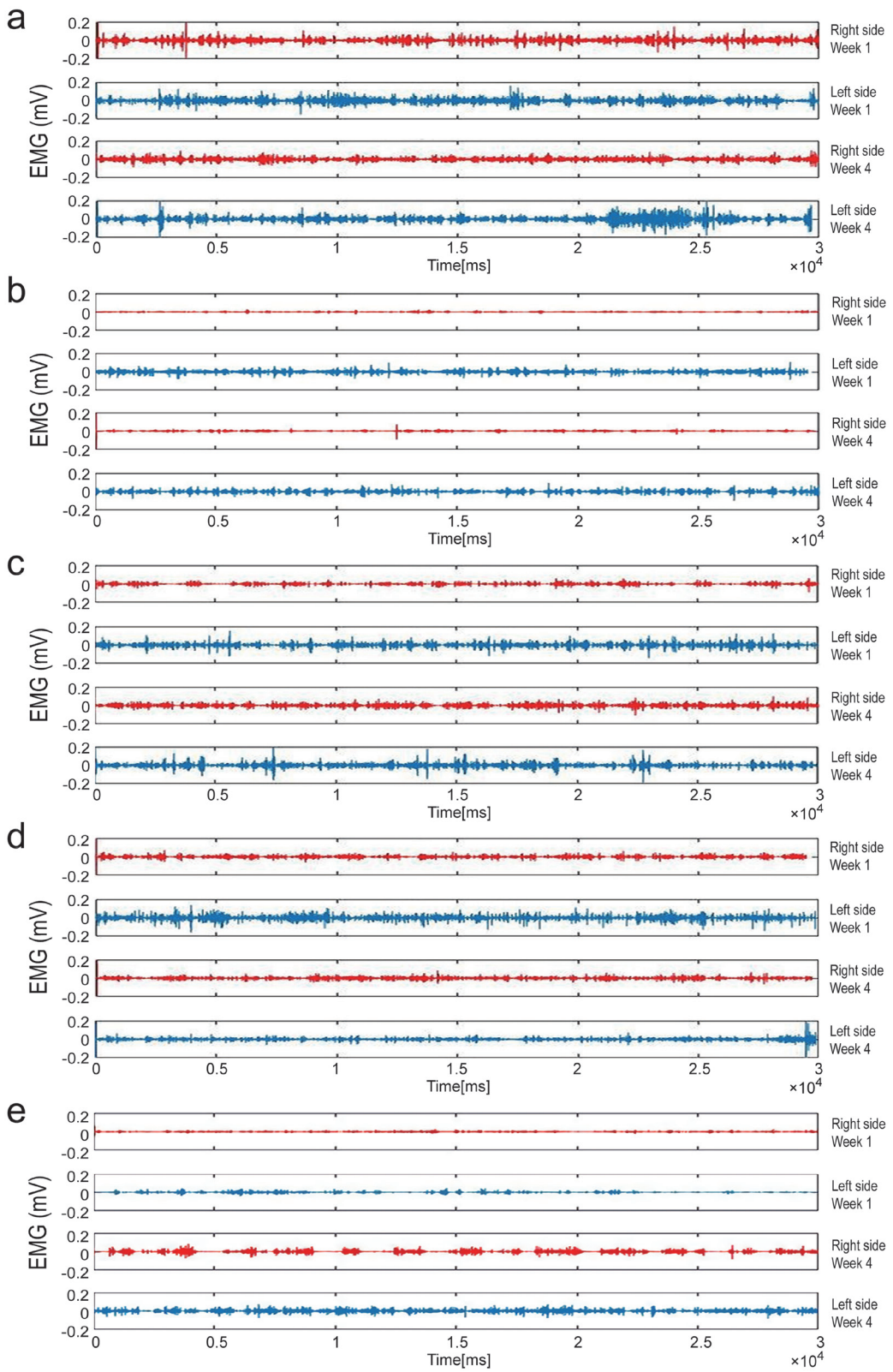

Fig. 3. The bilateal bicep EMG signal changes in rats in the different groups. (a) A rat in the sham operation group at the 1st and 4th week; (b) A rat in the denervation group at the 1st and 4th week; (c) A rat in the TNFR group at the 1st and 4th week; (d) A rat in the 2-week delayed TNFR group at the 1st and 4th week; (e) A rat in the 4-week delayed TNFR group at the 1st and 4th week. EMG, electromyography; TNFR, targeted neural function replacement.

Table 1. ARVs for active myoelectric remodeling of biceps brachii muscles in each group of rats

Group	Number of cases	First week	Fourth week	*p*
TNFR group	6	0.0050 ± 0.0002	0.0101 ± 0.0010[b]	<0.0001
2-week delayed TNFR group	6	0.0044 ± 0.0003	0.0091 ± 0.0008[b, c]	<0.0001
4-week delayed TNFR group	6	0.0038 ± 0.0004	0.0087 ± 0.0011[b]	<0.0001
Denervation group	6	0.0046 ± 0.0003	0.0051 ± 0.0006[a]	0.1529
Sham operation group	6	0.0089 ± 0.0004	0.0102 ± 0.0014[a]	0.0622

[a]Compared to the first week: $p > 0.05$; [b]Compared to the first week: $p < 0.05$; [c]Compared to the four week in the TNFR group, $p = 0.0259 < 0.0500$. ARV, average rectified value; TNFR, targeted neural function replacement.

affected by the extension of operation time. As a neural reconstruction technique, TNFR is based on the neural anastomosis theory. However, the nerve trunk to be reconstructed is thicker than the general neuromuscular branches, and efficiently achieving a functional connection with the target organ remains as a major challenge in surgical treatment.[15] In addition, end-to-end anastomosis is technically demanding for a surgeon, hindering the popularization of this technique.

According to the experiments by Marios *et al.*,[16] the reinnervation of autologous muscles can be reconstructed after nerve anastomosis surgery, because these can be successfully transferred during surgery to further restore function. However, compared to the use of intercostal nerve transfer for recovery, there is always a great risk of wasting the transferred motor nerve fibers into inappropriate channels. The more separated these are, the fewer the number of available motor fibers in the intercostal nerve. This is also one of the advantages of using the original nerve of the brachial plexus in this study.

In the field of human-computer interaction, the present study can provide basic EMG for amputees to control the intelligent prosthetic hand, and identify hand movement intentions. Combined with the fully connected layer structure, typical sampling, and standard regularization technology in deep neural networks, an improved multi-class neural fuzzy inference system can exist,[17] and its generalization ability has significantly improved, when compared to the traditional neural fuzzy inference system.

Limitations

In contrast to the report by Cage *et al.*,[18] the present study lacks high-density electroencephalography to localize the cortical activity associated with cued motor tasks generated by intact and absent limbs. Therefore, it remains unclear whether TNFR can restore normal cortical motor representations to control absent limbs. To improve the experiment, EMG can be added as one of the research directions for the recovery of missing limbs.

Future directions

Patients with physical disabilities have a high demand for multifunctional EMG prosthetics, but there are significant obstacles to its successful application in the case of insufficient residual EMG signals. Based on the surgical research of upper limb nerve function reconstruction and the extraction of its characteristic signal by wavelet packet reconstruction, a number of amputees in China are expected to make up for the deficiency of residual EMG signals with the help of the TNFR operation, achieving accurate control of the multifunctional EMG prosthesis. However, this needs an excellent sensor design, a more accurate control system, an appropriate action feature quantity, and a reasonable pattern recognition separator, in order to achieve the expected goal of improving the quality of life of disabled patients. In addition, a human-computer interface and application based on gesture recognition technology would be helpful in restoring their missing natural hand function through intelligent prostheses. Therefore, related research is essential for such vulnerable groups.[19]

Conclusions

The present results revealed that TNFR may be used well in rat neural function reconstruction, and can promote the establishment of a targeted neuromuscular network. In addition, neural function reconstruction in early TNFR operations would be better, and the ARV of the target muscle obtained after the operation would be enhanced. These preliminary research results obtained from rats can be used as an exploratory basis, in order to provide reliable experimental data for clinical treatment and post-amputation research, which may be helpful in improving the quality of life of amputee patients and their control of the prosthesis.

Supporting information

Supplementary material for this article is available at https://doi.org/10.14218/ERHM.2021.00036.

Supplementary File 1. ARRIVE checklist.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest concerning the materials or methods used in the study, or the findings specified in the article.

Author contributions

The authors' contributions to the study and manuscript preparation are, as follows: study supervision (YL and JZD), conception and design (YL), acquisition of data (WP, LYH and ZZJ), analysis and interpretation of data (ZZJ), drafting of the article (LYX and ZZJ), critical revision of the article (all authors), review of the submitted version of the manuscript and approval of the final version (all authors).

Ethical statement

The experimental animals were provided by the Animal Experimental Center of Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences (Experimental animal license no.: syxk [Yue] 2012-0119). This study was approved by the Animal Ethics Committee of Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences.

Data sharing statement

The technical appendix and relevant data are available from the corresponding author upon request: aiyzwll@aliyun.com.

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