A novel agonist-antagonist myoneural interface surgical approach on the proprioceptive reconstruction of rat lower extremity

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Research Article

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Abstract

Background

There is no consensus on the approach of agonist-antagonist myoneural interface (AMI) surgery for its effectiveness in repairing proprioception.

Objective

To investigate the effect of a novel AMI approach on proprioceptive reconstruction and motor repair of rat lower extremity.

Methods

Sprague-Dawley rats were randomly divided into AMI group and control group. AMI group rats were treated with the novel AMI surgical, which is characterized by the fixing of soleus muscle to the surface of biceps femoris muscle, following by anastomosing tibial nerve and common peroneal nerve to soleus muscles and suturing the two tendon terminals of soleus muscle. Control group rats were treated with the same process as AMI group except tendon terminal suture. Both electrophysiological, behavioral and immunohistochemical methods were applied to evaluate the difference.

Results

The functional index of sciatic nerve, tibial nerve and common peroneal nerve, as well as step angle and body angle in AMI group were significantly lower than control group after surgery ($P < 0.05$). The duration of walking swing, braking, propulsion and stance in AMI group were shorter than control group ($P < 0.05$). The mean pressure-touch intensity of the left paw in AMI group was less than control group. The nerve compound action potential (CNAP) of tibial nerve (common peroneal nerve) and muscle compound action potential (CMAP) of gastrocnemius muscle (tibialis anterior muscle) were stronger in AMI group.

Conclusion

These results suggested that the proposed surgical method can significantly improve the postoperative walking stability and muscle synergism in rats. In addition, due to the adoption of graft, donor selection avoids the limitation of nerve dissection condition, which can be extended to the whole body.

Introduction
High-energy injuries such as machine injuries, car accident injuries, and heavy object injuries caused by industrial construction accidents, transportation accidents, or natural disasters are increasing every year. These injuries not only cause severe fractures, extensive soft tissue defects, and large trauma contamination, but also may be accompanied by neurovascular injuries. Some patients may eventually have to undergo amputation. Myoelectric prostheses are one of the main types of prostheses used to restore limb function in amputees, which can transmit cortical motor signals to the motor nerve of the limb stump according to the patient’s needs, and then use surface electrodes to collect the nerve signals and control the prosthetic movement [1–6].

To achieve better control, Kuiken et al [7] first proposed the targeted muscle reinnervation (TMR) technique to transfer the amputated, which was used to control the movement of the prosthesis. Kung et al [8] also developed the latest method of regenerative peripheral nerve interface (RPNI), which can extract the efferent signal from the transected peripheral nerve and reproduce the afferent signal buried deep in the residual tissue, so that the prosthetic technology can realize the bidirectional feedback function with the peripheral nervous system [9–12]. The subset of afferent signals responsible for proprioception is still particularly difficult to capture in the above-mentioned models, and proprioception, including position, motion, and vibration sensation, cannot be reconstructed, and amputees basically have to rely on visual feedback to compensate for the lack of deep sensation to perform some movements [13–15]. The main reason why proprioceptive function has not been reconstructed is that none of these systems can provide physiologically relevant muscle-based proprioceptive feedback through natural neural pathways [16].

Proprioceptive impairment can severely affect motor control and balance and postural regulation, when conscious regulatory capacity is compensated for, leading to slow limb movements and abnormal gait [17–19]. In fact, for patients with lower extremity knee injuries, anterior cruciate ligament (ACL) reconstruction surgery is clinically advocated to preserve more joint ligament tissue [20–23]. This surgery is due to the presence of proprioceptors such as Ruffini and Paccini endings in the ACL, and only by preserving more proprioceptive information can the protective and stabilizing muscle reflexes and mechanical functions of the ACL be activated [24]. In order to make the rehabilitation of proprioceptive function more effective, Moezy et al [25] proposed whole body vibration training to replace conventional knee training, and Lin et al [26] proposed goal-matched walking training, both based on strengthening proprioceptive feedback connections at a later stage of functional reconstruction. All the above methods are beneficial for reconstructing proprioception in patients with proprioceptive damage such as knee injuries and ligament injuries, while in amputees with complete loss of muscles, nerves or even joints, the loss of effectors and receptors directly interrupts the proprioceptive feedback loop, and simple repair is clearly insufficient.

In 2017 Clites team proposed a method for proprioceptive construction, the agonist-antagonist myoneural interface (AMI) technique [27]. This technique directly connected two muscle tendons in series, one as the agonist muscle and one as the antagonist muscle, to build a synergistic muscle relationship to maintain the stretch of the agonist muscle by replicating the agonist-antagonist muscle-tendon pair, which was
effective in repairing proprioceptive feedback. This method mainly established tendon connections to adjacent residual muscles and directly sutures the tibialis anterior muscle to the gastrocnemius tendon to reform the proprioceptive loop system. Standard amputation surgery is designed mainly to fix the distal residual muscle tissue isometrically, with organs and other tissues distal to the amputation site discarded to provide filler for the residual distal end, while the distal end of the amputated nerve is usually buried in fatty tissue or deep in the residual limb to avoid its painful sensation due to mechanical stimulation [28–29]. However direct agonist-antagonist connection also has some limitations for patients with higher degrees of amputation, as there is little opportunity to establish muscle-tendon tissue connections due to excessive loss of muscle-tendon tissue, while tendon tissue at the distal end is more likely to be removed during amputation, thus limiting its application to patients with actual amputations. Second, the Clites team demonstrated the signal feedback loop problem mainly through the muscle force-electrical signal correlation, but the mechanism of reconstructing proprioception and postoperative lower limb muscle synergy in this surgical approach was not described in detail.

Herein, we proposed a graft-targeted tendon pair strategy to extend the selection of nerve implantation to the whole body, and to determine whether this approach can effectively repair the motor balance and stability of the lower extremity in rats by constructing an Sprague-Dawley (SD) rat lower extremity AMI model and analyzing the changes of proprioceptive information sources and effector signals. The rat soleus muscle with tendon tissue was selected as the donor, and the severed tibial nerve and common peroneal nerve were implanted in the soleus muscle belly, and the soleus tendon tissue was anastomosed end to end to form a complete anatomical closure of the nerve-muscle-tendon pair (Fig. 1). The novel AMI strategy builds on the RPNI technique by transplanting two target muscles as nerve bio-amplifiers while connecting the tendons to create the basic motor unit. One of the advantages of this approach is that the donor muscles are widely available, and the best donor can be arbitrarily selected according to the patient’s condition, while the patient is largely unlimited in the degree of amputation and can receive this surgical approach as long as the main functional nerve is preserved. In addition, due to the completion of the basic anatomical relationship that simulates the agonist-antagonist-tendon pair, when the tibial nerve signaling causes the contraction of the agonist muscle, it activates the agonist muscle proprioceptors, while the diastole of the coupled antagonist muscle activates its own receptors. Both then provide proprioceptive afferent signals through the tibial nerve and common peroneal nerve. This method is not only suitable for patients with a high degree of muscle tendon and nerve tissue loss, but also has stable nerve signal transmission. To verify whether this approach was effective, we established a rat amputation model with nerve dissection as a control and analyzed the postoperative myoelectric and neuroelectric signals from electrical signaling to visually demonstrate whether AMI surgery could directly connect the signal feedback chain by establishing morphological loops. In addition, we explored the mechanisms of proprioceptive repair using various biological indicators such as electrophysiological signaling, animal behavior, and histomorphology. The results suggested that AMI model was established by using soleus tendon pair grafting, which was feasible for constructing a closed loop of afferent and efferent signals from the agonist muscle-antagonist muscle and has a clear effect on the repair of
proprioception. Therefore, AMI surgery may become a new option for reconstructing proprioception of the missing limb in patients with disabilities.

**Figure 1** Agonist-antagonist myoneural interface (AMI) prosthetic model diagram: the soleus muscle was transplanted as the tibial and common peroneal nerve target muscles to establish an AMI model in SD rats, which has the advantage of being suitable for patients with severe amputation and an unlimited source of donor muscle. Since the agonist-antagonist muscle anatomical relationship is preserved, when the agonist muscle contracts, it can pull the antagonist muscle and induce proprioceptive signals, thus controlling the prosthetic movement.

**Methods**

**Animal model**

12 SD rats, male, weighing (450 ± 50) g [purchased from Guangdong Experimental Animal Center, animal permit number SCXK (Guangdong) 2017 – 0119], were housed in an SPF class animal room at (22 ± 2)°C, relative humidity 40%-60%, 12 h light and 12 h light avoidance cycle. The rats were randomly divided into AMI group and control group, with 6 rats in each group.

**Agonist-antagonist Myoneural Interface Surgery**

AMI group: isoflurane was used for general inhalation anesthesia, and a 1-cm incision was made along the heel tuberosity on the lateral side of the left and right lower limbs of SD rats. The subcutaneous tissues were separated layer by layer, and the fascia and connective tissues around the tendons of the distal ends of the two muscles were carefully peeled off and the distal soleus tendon was freed. The removed graft was immediately rinsed in sodium heparin solution (400 u/ml), removed excess blood and immersed in saline. The tibial nerve and common peroneal nerve were then separated and disconnected distally, and the proximal ends of the tibial nerve and common peroneal nerve were implanted at the belly of the two soleus muscles using a 12 – 0 surgical line. The soleus muscle was implanted at the surface fascia of the biceps femoris using an 8 – 0 surgical line, and the two soleus tendons were end-anastomosed using a 6 – 0 surgical line. After completion, they were sutured to the skin layer by layer.

Control group: the muscle graft and nerve implantation were performed as in the AMI group, until the two soleus muscles were implanted at the biceps femoris muscle membrane, and then the two muscles were only penetrated and slightly fixed with 6 – 0 surgical sutures, and the two tendons were not anastomosed.

**Testing Protocol**

**Gait analysis**
The gait analysis system (BT60101, Shenzhen Zhongshi Company, China) was used to record the postoperative footprints and gait of the rats. Three complete footprints were selected from the operated side and three from the non-operated side, and the distance between the heel and the tip of the foot, the first toe and the fifth toe, and the second toe and the fourth toe were measured. Simultaneous acquisition of walk body angle (angle between walking direction and trunk direction), swing duration, braking duration, propulsion duration, and stance duration.

**Characteristics of electromyographic signals and neuroelectrical information**

The hook-shaped microelectrodes were placed at the tibial nerve and common peroneal nerve respectively, and the stimulation signals were given by the Plexstim stimulator (OPX-D2, Plexon, USA) with different pulse widths of 50 µs and frequencies of 1 Hz. The muscle compound action potential (CMAP) of the tibial nerve innervated graft, the nerve compound action potential (CNAP) of the common peroneal nerve graft, and the corresponding tibial nerve and common peroneal nerve CNAP were collected using the microelectrodes. The Omniplex system software provided by Plexon was used to process the raw signals, and the CMAP and CNAP were pre-processed by filtering and noise reduction to extract the signals in the effective frequency domain band. The signal bands corresponding to the sensory stimuli were obtained after feature classification, and the spectra and peaks were further derived. The correspondence between the stimulus-muscle signal and the nerve signal is established.

**Estimation of the number of motor units**

The signal standard for different stimuli is to start with no contraction of the target muscle, and the contraction of the target muscle is gradually strengthened with the stimulus enhancement until the intensity remains unchanged. The stimulus intensity is recorded as IA, and then the stimulus is given once again, and the intensity IS = IA×(1 + 20%), and the electromyography (EMG) signal mEMG is counted under each stimulus intensity, and the number of motor units collected is calculated according to the linear relationship between mEMG amplitude and stimulus intensity.

**Body weight and muscle wet weight**

The body weights of the rats in the two groups were counted before and 2 months after surgery, and the gastrocnemius muscle (GAM), tibialis anterior muscle (TAM) and graft tendons were removed after anesthesia and weighed at 2 months after surgery.

**Histological staining**

The rat tendon pairs were removed intact and trimmed, fixed with 4% formaldehyde solution, then sequentially transparent, dehydrated, paraffin-embedded, routinely sectioned with a thickness of 5 µm, and sealed with neutral resin after staining. The morphology and pathological changes of skin tissue were observed microscopically. HE staining (BH0001, Boerfu Biotechnology, Inc., China) was used to observe the distribution and constructive characteristics of muscle spindles and tendons in the target area tissues, specifically the morphology of muscle myocytes, the number and morphology of tendons.
and spindles, the growth of blood vessels, and the healing of anastomosis, and Masson staining (BH0002, Boerfu Biotechnology, Inc., China) was used to observe the degree of tendon fibrosis.

**Western-blot detection of matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of metalloproteinases-1 (TIMP-1) expression**

20 mg of gastrocnemious tendon, tibialis anterior tendon and graft tendon tissues were taken from AMI group and control group respectively. The total tendon protein was extracted by using RIPA solution after freezing and breaking the tissues at -50°C using frozen tissue crusher, and the protein concentration was calculated by BCA method, separated by 100 g/L SDS-PAGE, electrotransferred to PVDF membrane, closed for 1h with closure solution and then washed. Mouse anti-rat TIMP-1 antibody (1:1000, C2321, Santa Cruz Biotechnology, Inc., USA), mouse anti-mouse MMP-1 antibody (1:1000, F0319, Santa Cruz Biotechnology, Inc., USA) and internal reference mouse anti-rat β-actin (1:1000, 123020210406, Beyotime Biotechnology, Inc., China) were added, incubated overnight at 4°C, washed 3 times. Horseradish peroxidase-edited goat anti-mouse IgG (1:1000, B1021, Santa Cruz Biotechnology, Inc., USA) was added. After incubation for 1h on a shaking bed and washing 3 times, the images were developed using ultrasensitive ECL. The grayscale values of the hormone tri-protein bands were calculated using Image J software, and the ratio of the grayscale values of TIMP-1 and MMP-1 to the grayscale value of the internal reference β-actin was calculated as the relative molecular expression of each target protein.

**Statistical analysis**

Statistical analysis was performed with SPSS22.0 software, and the measurement data such as sciatic nerve function index, MMP-1 and TIMP-1 expression were expressed as mean ± standard deviation, and the repeated measures ANOVA was used to test the repeated measures, and the LSD-t test was used for two-by-two comparison, and the difference between every two groups of the same index was tested by independent sample t test respectively. If the measurement data did not conform to normal distribution, the normality transformation was required, and then the differences between the data were tested. The above test was α = 0.05. The test should be adjusted for two comparisons of multiple groups, α' = α / k × (k – 1) / 2.

**Results**

**Gait analysis**

The absolute values of sciatic nerve function index, tibial nerve function index and common peroneal nerve function index, as well as paw angle (PA), Vector, paw angle body axis (PABA) of the gait angle values were significantly lower in the AMI group than in the control group 2 months after surgery, and the differences were statistically significant. The duration of walking swing, braking, propulsion and stance were shorter in the AMI group than in the control group, and the differences were statistically significant (P < 0.05). The mean pressure-touch intensity of the left paw in both groups was less than that of the
right hind foot, and the mean pressure-touch intensity of the left paw in the AMI group was less than that of the control group, while the mean pressure-touch intensity of the left-to-right walking process in the AMI group was less than that of the control group, and the differences were statistically significant, as shown in Fig. 2.

**Figure 2** Gait analysis data after surgery. A The atrophy of the affected foot in the AMI group (above) compared to the normal foot was obvious, while the atrophy in the control group (below) was more severe; B The absolute values of SFI, TFI and PFI in the AMI group (red) were significantly smaller than those in the control group (blue); C The angle between the walking direction and the carcass of the rats in the AMI group (above) was smaller, while the control group (below) was more deviated towards the affected foot; D The gait angle and body angle of the rats in the AMI group (red) were smaller than those in the control group (blue); E The pressure of the affected limb in the AMI group (above) was unstable during walking, with a brief lifting movement, while the control group (below) showed a continuous decrease in pressure; F The contact area and pressure of the plantar surface of the affected limb of the rats in the AMI group (above) was slightly weaker than that of the normal side, while the contact area of the plantar surface of the affected limb of the control group (below) was very small; G The duration of walking swing, braking, propulsion and stance of the rats in the AMI group (red) were all smaller than those in the control group (blue); H The pressure strength of the left foot in contact with the plane of the rats in the AMI group (red) were all smaller than those in the control group (blue), and the mean pressure-touch intensity of the left paw in the AMI group was less than that of the control group, while the mean pressure-touch intensity of the left-to-right walking process in the AMI group was less than that of the control group.

**Cmap And Cnap**

When the left common peroneal nerve was stimulated in the AMI group, the CMAP of the graft on the innervated side of the common peroneal nerve, the CMAP of the graft on the innervated side of the tibial nerve and the CNAP of the tibial nerve were all stronger, and the CMAP of the graft and the CNAP of the tibial nerve were gradually enhanced as the stimulation intensity increased (Fig. 2). In the control group, only the CMAP of the graft on the innervated side of the peroneal nerve could be recorded, and the CMAP of the graft on the innervated side of the tibial nerve and the CNAP of the tibial nerve were weaker; in the AMI group, the CMAP of the graft on the innervated side of the peroneal nerve, the CMAP of the graft on the innervated side of the tibial nerve and the CNAP of the peroneal nerve were stronger. When the stimulation intensity was gradually increased, the number of motor units recruited by the common peroneal nerve in the AMI group was not significantly different from that in the control group, while the number of motor units recruited by the tibial nerve was significantly higher than that in the control group, as shown in Fig. 3.

**Figure 3** Surgical schematic and signal analysis. A Electrical stimulation of the tibial nerve (agonist), recording of CMAP of the soleus muscle (agonist-antagonist) and CNAP of the common peroneal nerve
(antagonist); B Comparison of graft CMAP with CNAP of the contralateral nerve after stimulation of the nerve in the AMI group (blue) and control group (brown); C CMAP/CNAP ratios evoked by giving different stimulus intensities; D The stimulation intensity was gradually increased to induce CMAP/CNAP; E The CMAP/CNAP of the tibial nerve was stimulated in the AMI group, where blue was the CMAP of the innervated side graft of the tibial nerve, red was the CMAP of the innervated side graft of the common peroneal nerve, and orange was the CNAP of the common peroneal nerve; F The CMAP/CNAP of the common peroneal nerve was stimulated in the AMI group, where blue was the CMAP of the innervated side graft of the common peroneal nerve, red was the CMAP of the innervated side graft of the tibial nerve, and orange was the CNAP of the tibial nerve; G The CMAP/CNAP of the tibial nerve was stimulated in the control group, where blue was the CMAP of the innervated side graft of the tibial nerve, red was the CMAP of the innervated side graft of the common peroneal nerve, and orange was the CNAP of the common peroneal nerve; H The CMAP/CNAP of the common peroneal nerve was stimulated in the control group, where blue was the CMAP of the innervated side graft of the common peroneal nerve, red was the CMAP of the innervated side graft of the tibial nerve, and orange was the CNAP of the tibial nerve.

**Body Weight And Muscle Weight**

The differences in body weight, graft mass, tibialis anterior and gastrocnemius muscle mass between the left and right lower limbs of the rats in both groups before and 2 months after surgery were not statistically significant ($P>0.05$), but the tibialis anterior and gastrocnemius muscle masses of the right lower limb were significantly higher than those of the left lower limb in the group, and the differences were statistically significant ($P<0.05$), as shown in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Weight</th>
<th>Graft mass</th>
<th>TAM(L)</th>
<th>TAM(R)</th>
<th>GAM(L)</th>
<th>GAM(R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI group</td>
<td>8</td>
<td>615.50 ± 56.26</td>
<td>0.13 ± 0.08</td>
<td>0.25 ± 0.04</td>
<td>1.12 ± 0.11*</td>
<td>0.79 ± 0.15</td>
<td>3.95 ± 0.29*</td>
</tr>
<tr>
<td>Control group</td>
<td>6</td>
<td>581.83 ± 61.33</td>
<td>0.15 ± 0.03</td>
<td>0.22 ± 0.06</td>
<td>1.07 ± 0.06*</td>
<td>0.78 ± 0.16</td>
<td>3.64 ± 0.38*</td>
</tr>
<tr>
<td>$t$</td>
<td></td>
<td>1.067</td>
<td>0.578</td>
<td>1.126</td>
<td>1.001</td>
<td>0.120</td>
<td>1.737</td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td>0.307</td>
<td>0.574</td>
<td>0.282</td>
<td>0.337</td>
<td>0.906</td>
<td>0.108</td>
</tr>
</tbody>
</table>

Note: "*" expression compared to the left, $P 0.05$; TAM means tibialis anterior muscle; GAM means gastrocnemius muscle

**Histological Staining**
The results of HE staining showed that the tendon pairs in the AMI group had complete healing and complete connection, and some muscle tissues were atrophied with few normal pikes, while the tendons and muscle tissues in the control group showed obvious atrophy and loss of normal morphology and structure; Masson staining showed that the collagen fibers of the tendons in the AMI group were structurally intact, while the collagen fibers of the tendons in the control group were scattered in a fragmented manner (Fig. 4).

**Figure 4** Histological staining. A HE staining of the control group (2X): single soleus muscle; B HE staining of the control group (10X): severe muscle atrophy with loss of normal tissue structure; C HE staining of the AMI group (2X): soleus muscle tendon pairs; D HE staining of the AMI group (10X): partial muscle atrophy with essentially intact structure; E Masson staining of the control group (2X): single soleus muscle; F Masson staining of the control group (10X): complete atrophy of the tendon, severe fattening and structural disorder; G Masson staining of the AMI group (2X): soleus muscle tendon pair; H Masson staining of the AMI group (10X): complete healing of the flounder muscle tendon pair tendon pair with intact connection.

**WB**

MMP-1 and TIMP-1 levels in the AMI group were significantly higher than those in the control group, and the differences were all statistically significant ($P<0.05$). The difference in MMP-1/TIMP-1 expression levels between the two groups was not statistically significant ($P>0.05$), as shown in Fig. 5.

**Figure 5** WB. A MMP-1, TIMP-1 protein blots of the operated side tendons of rats in both groups; B MMP-1 levels in the AMI group (red) were significantly higher than the control groups (blue), the AMI group TIMP-1 expression levels were higher than the control group, and there were no significant differences in MMP-1/TIMP-1 levels in the AMI and control groups.

**Discussion**

Human limbs not only have flexible and powerful motor functions, but also have sophisticated sensory functions. While the fingertips perceive information about the surrounding environment through superficial senses such as touch and pressure to issue commands to control limb activities, proprioceptive receptors such as muscles, tendons, and joint pikes and tendons regulate limb position and movement accuracy at all times through position, kinesthetic, and vibration senses [30–31]. However, conventional prostheses do not help amputees to perform precise movement commands, sense their surroundings, and perceive the position, speed, and direction of motion of the prosthesis in the same way as a normal limb [32].

Clite's team established the basic anatomical relationship of the agonist-antagonist myoneural by tandemly coupling the gastrocnemius-anterior tibialis muscle in mice, using an external device to provide a standard motor signal to the efferent nerve to contract the aortic muscle and activate the aortic proprioceptive receptors, while the coupled antagonist muscle diastole activates its own receptors, both
of which provide proprioceptive afferent signals via the tibial and common peroneal nerves [33–35]. By re-establishing this basic motor synergy between the agonist-antagonist myoneural pair, the AMI technique uses native tissue receptors to convert prosthetic sensory information related to muscle stretch and tension into neural signals, and uses existing flexible interface technologies and physiologically relevant proprioceptive feedback from natural neural pathways to restore deep sensory functions such as motion position and vibration perception in the limb to provide amputees with real limb sensation [36]. Therefore, in this study, we investigated the effect of proprioceptive rehabilitation in rats after AMI surgery by establishing an effective method for the reconstruction of proprioceptive function in the missing limb using an animal model of limb disability.

To address the loss of limb proprioceptive function due to the disruption of the connection between proprioceptive signals and receptors after amputation, this study used the microscopic nerve anastomosis method to create a rat model of AMI in which the tibial and common peroneal nerves of the left hind limb of rats were disarticulated, and the two disarticulated ends of the nerves were terminated at the ventral part of the transplanted soleus muscle, and the transplanted tendons were microscopically anastomosed, and after a 2-month repair period, electrophysiological methods, gait analysis and expression of TIMP-1 and MMP-1 in the tendon were used to evaluate the postoperative muscle synergistic effects in rats, thus verifying the success of the model. The results showed that the gastrocnemius and tibialis anterior muscles in the AMI group (tendon anastomosis) and the control group (tendon dissection) showed significant atrophy compared with the healthy side (right side), and there was no significant difference in the degree of atrophy between the two groups. The absolute values of sciatic nerve function index, tibial nerve function index and common peroneal nerve function index, as well as the values of gait angle and body angle in the AMI group were significantly lower than those in the control group 2 months after surgery, suggesting that the repair status of injured nerve function and gait balance ability in the AMI group were better than those in the control group.

The proprioceptive is divided into conscious and non-conscious dominance modalities. The conscious proprioceptive pathway is mainly controlled by the integrated motor sensory areas of the flat cortex of the brain [37]. The control of proprioception is divided into high-level management by visual feedback combined with the involvement of the central nervous system in integrated information collection and processing, secondary management by the cerebellum and vestibule. Intermediate management, and primary management, which is low-level motor management in the conditioned reflex motor mode [38]. It is divided into limb position sensation, muscle kinesthesia and weight-bearing sensation [39]. In contrast, patients with lower limb amputation have a major loss of sensory information related to processing limb position and spatial movement due to the absence of limb muscle and tendon tissue or the loss of muscle and tendon proprioceptive receptors [40]. The use of lower limb prostheses requires higher requirements for trunk balance, postural adjustment, and motor control, and the basic control of the lower limb motor system needs to satisfy at least five points: (1) vertical support by gravity; (2) balance of the center of gravity; (3) postural stability; (4) control of the foot trajectory; (5) reduced speed of information transmission to higher centers [41–45].
First, the sarcomere tissue distributed in the skeletal muscles of the limb mainly encodes the analysis of muscle length signals and muscle extension changes, and this fast-conducting afferent feedback plays a key role in postural control regulation by regulating joint mobility in response to extension reflexes, etc. The tendon shuttle structures at the ends of the tendons, on the other hand, perceive changes in limb weight-bearing to assist in pedestrian motor load domination to move the limb forward, and the afferent input of kinesthetic information is significant for limb movement phase and pattern switching [46–48]. This pattern of muscle movement regulation that provides information to the higher nervous system about the relative position and movement of relevant muscles and joints is very delicate, and the loss of receptors and effectors caused by the absence of tissues such as muscles and joints in prosthetic patients directly disrupts the reflex loop [49]. Second, walking is not an unconscious process, but requires both "low-level" control of muscle and tendon shuttle receptors and "high-level" cognitive control, which is even more difficult for prosthetic users in terms of motor executive function and attentional control under walking conditions [50].

The walking swing duration is the maintenance time before the hindfoot touches the ground, and usually the faster the walking speed, the shorter the swing duration, while pain and lower limb motor function limitation caused by muscle and joint diseases can cause the swing duration to be prolonged, and the lack of kinesthesia and position sense will further prolong the swing duration before the hindfoot touches the ground [51]. The hindfoot starts to contact the ground after the swing phase, and the braking duration is from this time until the stage of maximum contact area between the hindfoot and the ground, when the limb is in the stage of deceleration and control of standing posture, and the prolongation of this stage may indicate that the body needs longer time to precisely distribute and control the standing load to ensure the balance of the limb. The propulsion duration, which is the time required to continue the forward motion, is from the maximum contact area between the hindfoot and the ground to the next. The duration of propulsion, the time required to continue forward motion, is the time between the maximum contact area of the hind foot with the ground and the next swing phase, which is another accelerated motion, and the shorter time may indicate greater trunk strength and control [52]. The stance duration is the whole process of hindfoot-ground contact and is generally positively correlated with walking speed. There are many influencing factors related to hindfoot-ground contact intensity, and rat body weight, plantar area, ground friction, locomotor status, and limb control may increase or decrease contact intensity [53]. The data from this study showed that the walking swing duration, braking duration, propulsion duration, and stance duration were significantly shorter in rats undergoing AMI surgery than in rats with tendon dissection after 2 months, and this result suggests that the intact neurofeedback loop established by AMI is effective in repairing the motor control of the lower limb in rats.

The stimulation-evoked electrical signal experiment showed that the CMAP signal of the peroneal nerve innervated graft (agonist muscle) was gradually enhanced with increased stimulation of the common peroneal nerve (agonist nerve) in the rats given AMI. At the same time, the CMAP signal of the tibial nerve innervated graft (antagonist muscle) was enhanced, and the CNAP signal of the tibial nerve (antagonist nerve) was increased. The results indicated that the co-motor unit of muscle-tendon pair-nerve could form a closed loop to complete the feedback of afferent and efferent signals, and the antagonist muscle and
antagonist nerve of the control group with severed tendon had no obvious feedback effect on the stimulation signal. The motor unit of the common peroneal nerve conduction in the AMI group was significantly higher than that in the control group, indicating that the synergistic effect of the agonist and antagonist muscles of the lower limb was good after AMI surgery, and the motor function of the injured nerve was recovered significantly.

MMP-1 interacts with TIMP-1 to maintain the relative stability of tendon collagen, and together they promote extracellular interstitial collagen renewal, regulate tendon metabolism, and participate in the tendon remodeling process [54–55]. However, in this study, MMP-1 and TIMP-1 were lower in the control group than in the AMI group, and there was no significant difference between MMP-1/TIMP-1 and AMI group, which may be due to the fact that the dynamic regulation of MMP-1/TIMP-1 is more obvious in the early tendon repair. After 2 months postoperatively, the tendon had entered a chronic repair phase and MMP-1/TIMP-1 regulation did not continue to function. Therefore, later experiments could intermittently extract protein to observe the tendon repair process. Histological staining showed significant atrophy of both tendon and muscle tissue in the control group, with disrupted tissue structure leading to loss of function, whereas tendon healing in the AMI group was as expected, with intact collagen fiber structure in the AMI group as seen by Masson staining. This result indicated that this surgical approach was anatomically and morphologically feasible to reconstruct the basic motor relationship of the agonist-antagonist muscle.

**Conclusion**

It is feasible to repair the lower limb function of tibial nerve and common peroneal nerve in rats by transplanting normal muscle tendon tissues from other parts to build the basic motor unit of agonist-antagonist myoneural. This method can not only significantly improve the postoperative motor stability and muscle synergy in rats, but also break through the previous restriction that dissociated nerve implantation can only select the target organ nearby, without affecting EMG and ENG collection and extraction. However, there are still some defects in this study. First, this study has not been able to accurately extract individual proprioceptive signals from the composite ENG signals for reference, and it has not been able to analyze the effects of this model from brain area innervation for the cerebral sensory cortex; second, the analysis of proprioceptive receptors such as tendons and muscle shuttles has not been completed in this study; third, the construction method of this model is complex to construct, and the tendon repair process is slow, and whether the rehabilitation of lower limb motor function can be promoted by drugs, thermal stimulation, electrical stimulation, motor stimulation, etc. still needs to be investigated.

**Abbreviations**

AMI
agonist-antagonist myoneural interface
CNAP
nerve compound action potential
CMAP
muscle compound action potential
TMR
targeted muscle reinnervation
RPNI
regenerative peripheral nerve interface
ACL
anterior cruciate ligament
SD rat
Sprague-Dawley rat
EMG
electromyography
GAM
gastrocnemius muscle
TAM
tibialis anterior muscle
MMP-1
Western-blot detection of matrix metalloproteinase-1
TIMP-1
tissue inhibitor of metalloproteinases-1
PA
paw angle
PABA
paw angle body axis.

**Declarations**

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**Author contributions**

Ping Wang and Jianping Huang were responsible for the conception and design of the study. The data were analyzed by Ping Wang and Jianping Huang. Animal surgery and data collection were performed by Ping Wang, Jianping Huang, Jingjing Wei, Qianhengyuan Yu. Ping Wang drafted the manuscript. Zhiyuan Liu (corresponding author) and Lin Yang provided substantial input to the first draft. Guanglin Li provided intellectual input into the final draft of the manuscript. All authors reviewed the manuscript and gave final approval of the version to be submitted. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

There is no conflict of interest regarding the publication of this paper.

Ethics approval

All animal care and use procedures were conducted in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals. The experiment was approved by the Experimental Animal Ethics Committee of Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, under protocol number SIAT-IACUC-210907-JCS-HJP-A2050.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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**Figures**

![Figure 1](image)

**Figure 1**

Agonist-antagonist myoneural interface (AMI) prosthetic model diagram: the soleus muscle was transplanted as the tibial and common peroneal nerve target muscles to establish an AMI model in SD rats, which has the advantage of being suitable for patients with severe amputation and an unlimited source of donor muscle. Since the agonist-antagonist muscle anatomical relationship is preserved, when the agonist muscle contracts, it can pull the antagonist muscle and induce proprioceptive signals, thus controlling the prosthetic movement.
Figure 2

Gait analysis data after surgery. A The atrophy of the affected foot in the AMI group (above) compared to the normal foot was obvious, while the atrophy in the control group (below) was more severe; B The absolute values of SFI, TFI and PFI in the AMI group (red) were significantly smaller than those in the control group (blue); C The angle between the walking direction and the carcass of the rats in the AMI group (above) was smaller, while the control group (below) was more deviated towards the affected foot;
D The gait angle and body angle of the rats in the AMI group (red) were smaller than those in the control group (blue); E The pressure of the affected limb in the AMI group (above) was unstable during walking, with a brief lifting movement, while the control group (below) showed a continuous decrease in pressure; F The contact area and pressure of the plantar surface of the affected limb of the rats in the AMI group (above) was slightly weaker than that of the normal side, while the contact area of the plantar surface of the affected limb of the control group (below) was very small; G The duration of walking swing, braking, propulsion and stance of the rats in the AMI group (red) were all smaller than those in the control group (blue); H The pressure strength of the left foot in contact with the plane of the rats in the AMI group (red) were all smaller than those in the control group (blue), and the mean pressure-touch intensity of the left paw in the AMI group was less than that of the control group, while the mean pressure-touch intensity of the left-to-right walking process in the AMI group was less than that of the control group.
Figure 3

Surgical schematic and signal analysis. A Electrical stimulation of the tibial nerve (agonist), recording of CMAP of the soleus muscle (agonist-antagonist) and CNAP of the common peroneal nerve (antagonist); B Comparison of graft CMAP with CNAP of the contralateral nerve after stimulation of the nerve in the AMI group (blue) and control group (brown); C CMAP/CNAP ratios evoked by giving different stimulus intensities; D The stimulation intensity was gradually increased to induce CMAP/CNAP; E The
CMAP/CNAP of the tibial nerve was stimulated in the AMI group, where blue was the CMAP of the innervated side graft of the tibial nerve, red was the CMAP of the innervated side graft of the common peroneal nerve, and orange was the CNAP of the common peroneal nerve; **F**The CMAP/CNAP of the common peroneal nerve was stimulated in the AMI group, where blue was the CMAP of the innervated side graft of the common peroneal nerve, red was the CMAP of the innervated side graft of the tibial nerve, and orange was the CNAP of the tibial nerve; **G**The CMAP/CNAP of the tibial nerve was stimulated in the control group, where blue was the CMAP of the innervated side graft of the tibial nerve, red was the CMAP of the innervated side graft of the common peroneal nerve, and orange was the CNAP of the common peroneal nerve; **H**The CMAP/CNAP of the common peroneal nerve was stimulated in the control group, where blue was the CMAP of the innervated side graft of the common peroneal nerve, red was the CMAP of the innervated side graft of the tibial nerve, and orange was the CNAP of the tibial nerve.
Figure 4

Histological staining. A HE staining of the control group (2X): single soleus muscle; B HE staining of the control group (10X): severe muscle atrophy with loss of normal tissue structure; C HE staining of the AMI group (2X): soleus muscle tendon pairs; D HE staining of the AMI group (10X): partial muscle atrophy with essentially intact structure; E Masson staining of the control group (2X): single soleus muscle; F Masson staining of the control group (10X): complete atrophy of the tendon, severe fattening and
structural disorder; G Masson staining of the AMI group (2X): soleus muscle tendon pair; H Masson staining of the AMI group (10X): complete healing of the flounder muscle tendon pair tendon pair with intact connection.

Figure 5

WB. A MMP-1, TIMP-1 protein blots of the operated side tendons of rats in both groups; B MMP-1 levels in the AMI group (red) were significantly higher than the control groups (blue), the AMI group TIMP-1 expression levels were higher than the control group, and there were no significant differences in MMP-1/TIMP-1 levels in the AMI and control groups.