Development of less invasive neuromuscular electrical stimulation model for motor therapy in rodents

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Background: Combination therapy is essential for functional repairs of the spinal cord. Rehabilitative therapy can be considered as the key for reorganizing the nervous system after spinal cord regeneration therapy. Functional electrical stimulation has been used as a neuroprosthesis in quadriplegia and can be used for providing rehabilitative therapy to tap the capability for central nervous system reorganization after spinal cord regeneration therapy.

Objective: To develop a less invasive muscular electrical stimulation model capable of being combined with spinal cord regeneration therapy especially for motor therapy in the acute stage after spinal cord injury.

Methods: The tibialis anterior and gastrocnemius motor points were identified in intact anesthetized adult female Fischer rats, and stimulation needle electrodes were percutaneously inserted into these points. Threshold currents for visual twitches were obtained upon stimulation using pulses of 75 or 8 kHz for 200 ms. Biphasic pulse widths of 20, 40, 80, 100, 300, and 500 μ s per phase were used to determine strength–duration curves. Using these parameters and previously obtained locomotor electromyogram data, stimulations were performed on bilateral joint muscle pairs to produce reciprocal flexion/extension movements of the ankle for 15 minutes while three-dimensional joint kinematics were assessed.

Results: Rhythmic muscular electrical stimulation with needle electrodes was successfully done, but decreased range of motion (ROM) over time. High-frequency and high-amplitude stimulation was also shown to be effective in alleviating decreases in ROM due to muscle fatigue.

Conclusions: This model will be useful for investigating the ability of rhythmic muscular electrical stimulation therapy to promote motor recovery, in addition to the efficacy of combining treatments with spinal cord regeneration therapy after spinal cord injuries.

Keywords: Spinal cord injuries, Neuromuscular electrical stimulation, Activity-based therapy, Rehabilitation, Rat, Kinematics, Motor therapy, Neuroregeneration, Neuroprotection

Introduction

In contrast to incomplete spinal cord injuries, recovery of motor function in cases of complete spinal cord injuries is generally limited,¹ and cell transplantation as well as other types of regenerative therapies need to be investigated. At present, neuroprotection, axonal growth stimulation, lost tissue replacement, and axonal transmission enhancement are areas of research, but since each of these treatments has its limitations when used alone combining them may be beneficial.² Stimulation

of the intrinsic plasticity of the nervous system via rehabilitation may improve functional recovery after incomplete spinal cord injuries, and it is hoped that rehabilitation can stimulate the reorganization of neural circuits after regenerative therapies.² In people with spinal cord injuries, repeated rhythmical exercise of the legs together with robot-assisted walking on a treadmill and functional electric stimulation therapy are believed to be clinically effective.^{3–5} These improvements appear to be task specific and rely on sensory feedback mechanisms.^{6,7}

Electrical stimulation has been reported to be effective in actual clinical cases when not only used as a

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method to restore function,^{8–12} but also as a method to train motor functions.^{4,13–15} Reorganization of the neural network of the central nervous system that results from rhythmical exercises has been cited as a mechanism that can improve motor function,^{5,16,17} but the details of this mechanism remain unclear.¹⁸ To understand the details of these mechanisms and investigate their effectiveness when combined with spinal cord regeneration therapies, basic research, including animal experiments, is necessary. However, since there are very few reports on the effectiveness of combined spinal cord regeneration therapies based on animal models, this area requires further study, including the development of experimental animal models.^{2,19–21}

Jung *et al.*²² have previously created neuromuscular electric stimulation (NMES) models using rats, stimulated the motor points of the major joints of the limbs using embedded electrodes²³, and reported significant short-term improvements in hindlimb synkinesia of rats with incomplete spinal cord injuries after NMES therapy of only hip joints.²⁴ Electrical stimulation of muscles for motor therapy would be able to start from acute stage after spinal cord injury or regeneration therapy of spinal cord injury, and it could become useful rehabilitative strategy in that stage. The use of intramuscular electrodes is highly invasive for rats after spinal cord injury because it requires long and invasive surgery and has many complications, such as electrode infection, chewing, etc. Thus, a less invasive method is very desirable to study the effectiveness of combination of spinal regeneration therapies. Surface electrodes are noninvasive and clinically may be better option for therapy application. In rats, it is difficult to stimulate continuously the motor points of muscles during motor therapy, because the skin of rats is very flexible. Thus, we used needle electrodes, which are comparatively less invasive than intramuscular electrodes in this study (Table 1). Our long-term goal is to create a less invasive electrical stimulation therapy model that can be used together with spinal cord regeneration therapies and to investigate its effectiveness for motor therapy especially in the acute stage after spinal cord injury.

This study tested the hypothesis that a muscular electrical stimulation therapy model that used needle

electrodes instead of intramuscular electrodes can produce rhythmic movement of ankle joints in normal rats under the same stimulation parameters used in previous studies.^{23,24} Jung *et al.*²⁴ reported the rapid range of motion (ROM) decreasing related to muscle fatigue. The maintenance of ROM may produce much more sensory feedback that could lead to be more functional recovery.^{6,7} Ward and co-workers^{25,26} reported the potential usefulness of kHz frequency alternating current for rapid muscle fatigue-associated functional electrical stimulation. In this study, we also tested the effect of kHz frequency for muscle fatigue associated with electrical stimulation.

Materials and methods

Animals

This study was approved by the Committee for the Care and Use of Animals at the Yamaguchi University. Ten mature female Fischer rats (160–175 g) were used. Needle electrodes were inserted in the tibialis anterior muscle (TA) (ankle flexor) and gastrocnemius muscle (Gc) (ankle extensor). An inhalation anesthetic (2–3% Sevoflurane) was used to anesthetize the rats.

Insertion of stimulation electrodes

Stimulation electrodes (TECA Disposable Monopolar Needle Electrode 902-DMF25-TP, Carefusion Japan, Tokyo, Japan) with diameters of 0.30 mm and a recording area of 0.03 mm² were inserted while the rats were anesthetized. The rats were secured to a custom-made platform and electrodes were percutaneously inserted in TA and Gc on both hindlimbs of the animal. In order to insert the electrodes near the motor points of each muscle, anatomical motor points were referenced (Fig. 1) and electrodes were inserted at the point of greatest contraction after percutaneous stimulation of the target muscle using a low current (frequency, 75 Hz; pulse width, 40 μ s; amplitude, 1 mA; and duration, 200 ms).

Strength–duration curve

To assess whether the stimulation electrodes were positioned at the appropriate points near the motor points of the target muscles, after electrode positioning strength–duration (SD) tests were conducted for all

Table 1 Invasiveness of needle electrodes and their potential application

	Noninvasive	Minimally invasive	NMES application	Therapy application
Surface electrodes	○		○	○
Needle electrodes		○		○
Implanted electrodes			○	○

Figure 1 Motor point anatomy of targeted hindlimb muscles and inserting procedures of stimulating electrodes. Detailed motor point anatomy of targeted hindlimb muscles is shown in (A) (TA) and (B) (Gc). In TA (A and C), deep peroneal nerve goes to TA motor point (A ★: 3 mm in depth from the point which is 5 mm below and 5 mm anterior to FH) through just below the fibular head (FH), and it is possible to get the motor point by inserting the needle electrode from just below the FH to TA (C ☆). In Gc (B and D), tibial nerve goes to Gc motor point (B : posterior proximal center of lower hindlimb at level of 5 mm below FH), which is proximal center between GL and GM, at same level of deep peroneal nerve, and it is possible to get the motor point by inserting the needle electrode from the posterior proximal center of the lower hindlimb to GM (D ☆). TA, tibialis anterior; Gc, gastrocnemius; GL, gastrocnemius lateralis; GM, gastrocnemius medialis; ST, semitendinosus; TT, tibial tuberosity; FH, fibular head; BFk, bicepsfemoris, knee flexion. ★ ☆, motor point; ▲, landmark.

muscles being studied. In this study, twitch threshold currents were determined for different pulse durations (20, 40, 60, 100, 200, and 500 μ s).

The rheobase (twitch threshold current at very long pulse duration (here 500 μ s) and a range of chronaxie (stimulus duration at the point where the twitch threshold current is twice the rheobase) were determined to assess the excitability of the muscle from the SD curves.

NMES parameters

An isolated four-channel stimulator[®] (STG2004, Multi Channel Systems, Cytocentrics, Rostock, Germany) was used in this study. In Group A ($n = 5$), the amplitude of the stimulation current was set to 1.5 times the threshold known to produce visual twitches at a pulse width of 40 μ s and a frequency of 75 Hz obtained

from the SD curves.^{23–25} Jung *et al.*²⁴ reported the rapid ROM decreasing related to muscle fatigue for 15 minutes NMES. Ward and co-workers^{26,27} reported the potential usefulness of kHz frequency alternating current for rapid muscle fatigue associated functional electrical stimulation. They^{28,29} also reported that a frequency close to 10 kHz is indicated for comfortable motor stimulation, and frequencies above 10 kHz have little or no useful clinical role in rehabilitation procedures. To investigate the usefulness of kHz frequency stimulation for the rapid ROM decreasing related to muscle fatigue, another stimulation pattern with different frequencies and amplitudes was used in this study. In Group B ($n = 5$), the amplitude of the stimulation current was set to three times the threshold known to produce visual twitches at a pulse width of 40 μ s and

frequency of 8 kHz (maximum frequency setting in this stimulator system) obtained from the SD curves. The interval between stimulations was set at 0.6 seconds, and the duration of the stimulations was 84 ms at TA and 240 ms at Gc. The timing of the stimulations of the muscles was calibrated in reference to previous electromyogram data obtained from treadmill experiments of normal rats.³⁰ Stimulations were performed for 15 minutes while the rats were anesthetized.

Three-dimensional kinematic analysis

To evaluate sequential changes in the articular ROM of the stimulated ankle joints, we performed three-dimensional (3D) kinematic analysis using KinemaTracer® (Kissei Comtec Co., Ltd, Nagano, Japan). Colour markers were attached to the joints of both hindlimbs (at the surface of the skin at the iliac region and at the hip, knee, ankle, and metacarpophalangeal joints). Four CCD video cameras were used to film these markers. Using the kinematic analysis software, the

ankle joint ROM was calculated and sequential changes were evaluated immediately, 5, 10, and 15 minutes after stimulation (Fig. 2).

Statistical analysis

The paired *t*-test was used to compare sequential changes in ankle ROM and repeated measure analysis of variance was used to compare the two groups. Differences were considered significant based on a hazard ratio of 5% using Excel.

Results

SD curves

Typical SD curves of left TA of Groups A and B were shown in Fig. 3. The curves show a typical nonlinear hyperbolic relationship in which stimulation pulses with shorter pulse widths require higher current amplitude to reach the threshold.

The average rheobase value of each group was calculated to be 0.11 ± 0.06 mA (Group A, $n = 20$) and

Figure 2 3D kinematic analysis. Color markers (●) (B) were attached to the limbs and photographed using four CCD cameras (○) (A). 3D motion analysis was performed to evaluate sequential changes in ROM of ankle joints of both hindlimbs during stimulations.

Figure 3 SD curves of left TA muscles of Groups A and B. Typical SD curves of left TA muscles in Groups A (A) and B (B) are shown. Both SD curves show a typical nonlinear hyperbolic relationship in which stimulation pulses with shorter pulse widths require higher current amplitude to reach the threshold.

Table 2 Average rheobase and chronaxie values of each muscle in Groups A and B

	Left TA	Right TA	Left Gc	Right Gc
Group A (<i>n</i> = 20)				
Rheobase (mA)	0.9 ± 0.02	1.2 ± 0.05	0.9 ± 0.02	1.5 ± 0.09
Chronaxie (μs)	142 ± 62	120 ± 43	148 ± 54	132 ± 54
Group B (<i>n</i> = 20)				
Rheobase	1.1 ± 0.07	2.2 ± 0.1	1.2 ± 0.06	1.7 ± 0.08
Chronaxie	102 ± 56	66 ± 15	72 ± 26	68 ± 8.4

Values are mean ± SE.

0.16 ± 0.03 mA (Group B, *n* = 20), and the average chronaxie value was 135 ± 50 μs (Group A) and 77 ± 33 μs (Group B) (average rheobase and chronaxie values of each muscle are shown in Table 2).

3D kinematic analysis

Fig. 4 shows the sequential changes observed in the average ROM of both ankles of Group A and B rats. The initial average ROM of each group was calculated to be 32 ± 9° for Group A and 61 ± 8° for Group B. In both Groups A and B, a significant decrease in ROM was observed at 5 minutes after the onset of stimuli (*P* = 0.001, Group A; *P* = 0.00002, Group B). In comparison with Group A, ROM of Group B was significantly larger (*P* = 0.0006) at all time points measured, and an interaction between stimulation parameters and period after stimulation (*P* = 0.001) was observed. Fig. 5 shows the stick figure and the average angles of the ankle joints of Group A rat during one stimulation cycle (100%). From the ankle joint paths, we confirmed a joint movement during walking at the timing of walking stimulation.

Figure 4 Sequential changes in average ankle joint ROM (Groups A and B). In both Groups A and B, a significant decrease in ROM was observed at 5 minutes after the onset of stimuli. In comparison with Group A, ROM of Group B was significantly larger (*P* = 0.0006) at all time points measured, and an interaction between stimulation parameters and period after stimulation (*P* = 0.001) was observed.

Discussion

To our knowledge, this is the first study to use needle electrodes applicable to rhythmic electrical stimulation of muscles for motor therapy in rats. A problem that emerges when performing NMES with needle electrodes is the placement of electrodes near the motor points of the target muscles. Stimulation sites closer to the motor point require lower charge levels for muscle activation. Ichihara *et al.*²³ reported detailed anatomical motor points of rat hindlimb muscles. The motor points of TA and Gc are relatively easy to stimulate with needle electrodes, and thus were used in this study (Fig. 1). Crago *et al.*³¹ reported that the rheobase was 5.2 mA and the chronaxie 85 μs for intramuscular electrodes implanted in the TA of the cat; for electrodes implanted near the peroneal nerve, the rheobase was identified to be 0.38 mA and chronaxie 55 μs. In studies of intramuscular electrodes implanted in rat hindlimb muscles, the rheobase was reported to be 0.16 mA and the chronaxie values ranged from 40 to 90 μs. In this study, we were able to achieve low rheobase currents (0.11 mA, Group A; 0.16 mA, Group B). The average chronaxie value of Group A (135 μs) was higher than Group B (77 μs), that would be attributable to the higher stimulus frequencies of Group B. From these data, we confirmed that the stimulation electrodes were inserted at the appropriate sites near the motor points (Table 2, Fig. 3).

Rhythmic exercises like locomotion can improve motor function in cerebral infarction¹⁴ or spinal cord injury,¹⁵ and electrical stimulation could modify spinal and cortical neural circuitry.¹⁸ To generate locomotor-type movement patterns by electrical stimulation, it is necessary to obtain sufficient angular excursion during electrically stimulated movements. The ROM (flexion plus extension) of the ankle joint was reported to be approximately 65° in 3D kinematic analysis during treadmill walking for rats.³⁰ Ichihara *et al.*²³ reported that they were able to achieve a total excursion of 70° at ankle joint (35° for flexion and 35 for extension) using NMES (multiple pulse stimulation at 100 Hz, pulse widths of 40 μs, and current amplitude of 1.5

Figure 5 Stick figure and paths of average ankle joint angles on both sides during stimulation. The graph (B) shows (Group A, rat no. 143) one cycle of stimulation (100%) immediately after stimulation and the average angle paths of both ankle joints for 11 seconds captured on CCD cameras. The upper two graphs show the paths of the ankle joint angles (the top shows the right ankle joint angle; the second from the top shows the left ankle joint angle) and the bottom four graphs show the stimulation timings and current strengths (CH_1: left TA; CH_2: left Gc; CH_3: right TA; and CH_4: right Gc). The graphs show that good walking joint movement was obtained from the timing of the walking stimulation of both the left and right ankle angle paths. Stick figure (A) shows only ankle joint movement of bilateral hindlimbs during stimulation.

times the twitch threshold current at 40 μ s). In this study, the initial average ROM of Group A was relatively small ($32 \pm 9^\circ$), but that of Group B was sufficient for locomotor-type movement of the ankle joint ($61 \pm 8^\circ$). Ankle joint ROM of this study was smaller than that of previous study²³ especially in Group A, because they stimulated each muscle once about 2.5 seconds and calculated each joint ROM which was not rhythmic stimulation of ankle muscle pairs in this study.

Jung *et al.*²⁴ observed that when using intramuscular electrodes in hip joint agonists, ROM decreases significantly within a minute of stimulation. In this study, we performed electrical stimulation of muscles under stimulation parameters identical to those used by Jung *et al.*²⁴ While this study was successfully able to utilize cyclic electrical stimulation using needle electrodes in different muscles (Fig. 5), ROM significantly decreased at 5 minutes after stimulation (Fig. 4). Because this was an open-loop stimulation, it was assumed that the stimulated muscles would fatigue.

In previous studies,^{32,33} higher intensity and frequency generate stronger muscle contraction, but also a stronger decline in force and thus quick setting muscle fatigue; nevertheless, most of them were based on the results from continuous electrical stimulation. There were several reports on the study of intermittent electrical stimulation.^{34–36} Matsunaga *et al.*³⁵ and Shimada *et al.*³⁶ showed that high-frequency electrical stimulation elicits less fatigue than low-frequency electrical stimulation during a repetitive stimulation

protocol. Dreibati *et al.*³⁴ reported that only high-frequency (100 Hz) intermittent electrical stimulation within 5 minutes met the criteria of appropriate muscle force training. Ward and co-workers^{26,27} reported the potential usefulness of kHz frequency alternating current for rapid muscle fatigue associated functional electrical stimulation. They^{28,29} also reported that a frequency close to 10 kHz is indicated for maximum comfort with low torque, and a frequency of 1 kHz or less is preferable for maximum torque.

To assess the impact of electrical stimulation frequency and intensity on muscle force and fatigue, the amplitude of the stimulation current was set to three times the threshold known to produce visual twitches at a pulse width of 40 μ s and frequency of 8 kHz. A significant decrease in ROM was observed at 5 minutes after stimulation in Group B, but in comparison with Group A ROM was larger in Group B at all time points ($P = 0.0006$) (Fig. 5). ROM was maintained in Group B, and based on the fact that interactions exist between the stimulus parameters and period after stimulation ($P = 0.001$), it can be inferred that it is possible to counteract decreases in ROM due to muscle fatigue by using high-amplitude and high-frequency stimulation currents within 15 minutes in this study. Therefore, it is possible to maintain ROM by using a stimulus program in which both frequency and current are increased incrementally during stimulation. Nevertheless, stimulation frequency used in Group B is very high (8 kHz), and there is no report of kHz frequency electrical stimulation

for locomotor training. It is essential, therefore, to identify appropriate frequency and amplitude of NMES to counteract muscle fatigue in the future.

Other methods for maintaining ROM have also been previously reported. Fairchild *et al.*³⁷ successfully utilized adaptive NMES with intramuscular electrodes embedded in the hip joint of rats with spinal cord injuries.³⁷ They used a pattern generator/pattern shaper closed-loop stimulator – which maintains ROM by using real-time feedback of the hip joint angle during stimulation to set the ideal hip joint motion path in advance – to automatically adjust the current pulse amplitude and correct errors. Using this type of stimulation algorithms in the future should be able to solve the problem of muscle fatigue encountered in this study. As potential limitations of this electrical stimulation of muscles for motor therapy, the movements produced by the stimulation of only ankle joint muscles cannot provide a replacement for locomotion involving all of the muscles normally active during walking movements. The combination therapy, such as body weight support treadmill training, could be necessary for more effective functional recovery.⁴ The needle electrodes used in this study cannot replace surface electrodes as clinical NMES application (Table 1), but the needle electrode could have a potential usefulness as a tool for peripheral nerve stimulation, that may modulate plasticity in the spinal cord.^{7,16,38}

Conclusion

In summary, a less invasive muscular electrical stimulation model for motor therapy was developed using needle electrodes. SD curves were used to confirm that the stimulation electrodes were inserted at the appropriate sites. Rhythmic stimulation of the leg joints using muscular electrical stimulation with needle electrodes was successful, but evaluation of sequential ROM using 3D kinematic analysis showed that at 5 minutes after stimulation, a significant decrease in ROM was observed. High-frequency and high-amplitude stimulation was also shown to be effective in alleviating decreases in ROM due to muscle fatigue. In comparison with NMES therapy rodent models that use intramuscular electrodes, this model is less invasive and can be used in combination with spinal regeneration therapies from the acute stage.

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