Myoelectrical activity and muscle morphology in a rat model of myofascial trigger points induced by blunt trauma to the vastus medialis

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ABSTRACT

Objectives To explore myoelectrical activity and muscle morphology of myofascial trigger points (MTrPs) in an injury model of rats.

Methods A total of 24 male SD rats were randomly divided into a control group (group A) and model group (group B). A blunt striking injury and eccentric exercise were applied to the vastus medialis (VM) of rats in group B for 8 weeks. Later, the palpable taut band (TB), local twitch response, myoelectrical activities and morphology in the two groups were examined.

Results An average of 2.5 (30/12) palpable TBs were detected in the VM in group B compared with none in group A. The MTrPs had two types of abnormal potential. Their amplitudes were significantly higher than those in the control group (p<0.01) but their durations showed no significant differences. A series of reflex contractions appeared in groups A and B in response to external stimulation to the ear. Their amplitude and duration in group B were significantly lower than those in group A. A series of lower fibrillation potentials repeatedly occurred in model MTrPs in group B. The morphology of MTrPs showed abnormal muscle fibres with large round or ellipse shapes in cross-section and enlarged tapering shapes in longitudinal section.

Conclusions Active MTrPs can be provoked by repeated blunt injury. Active MTrPs are a group of muscle fibres with abnormal shapes and abnormal myoelectrical potentials. External stimulation provokes low-voltage responses in MTrPs, which is different from the response of normal muscle fibres.

INTRODUCTION

The injuries of daily life activities and sports can lead to pain and local motor dysfunction. Sometimes, the pain does not indicate local damage but rather the activation of myofascial trigger points (MTrPs) in muscles because of the original trauma or certain other factors. The syndrome includes local referred pain and a group of other symptoms. Hubbard and Berkoff found that MTrPs in patients with neck and shoulder pain had spontaneous action potentials with two forms: continuous low-voltage waves and some sporadic high-voltage sharp spikes.1 Fricton et al2 observed tall spike potentials in needling electromyography (EMG) in human MTrPs. Hong et al3 first found a latent MTrP in the biceps femoris of rabbits, in which the MTrP had the characteristics of a twitch response, taut band (TB) and spontaneous electromyographic activity (SEA). In most studies, animal models of MTrPs are generated by the injection of acetylcholine and/or other chemical agents into the animal muscle.4–8 A reliable model of MTrPs based on direct injury of animals has not yet been reported. However, injury is one of the main factors that causes MTrPs in clinical practice. Therefore, an injury MTrP model is needed.

Employing a repeated eccentric exercise with load to the human finger, Kawakitah et al obtained an acute MTrP in the extensor digitorum with SEA, but it quickly disappeared in a few days.9 Based on the local injuries, a model of MTrPs in rats was established with SEA by Yao and Huang.10 Combining a local strike with eccentric exercises, Han et al11 found large circular muscle fibres in the region of the TB, in which regional SEA was recorded. From their results, MTrPs were confirmed in the thigh muscles of rats. Following these previous
studies, the purpose of this study is to further develop a model of MT rPs in rat muscle through extending the traumatic and resting times to a possible chronic status. Using this model, the myoelectrical potentials and morphology of MT rPs will be observable and can be analysed.

METHODS

Animals
A total of 24 male SD rats were used; mean age and body weight were 7 weeks old and 220–250 g, respectively. The rats were randomly divided into two groups: a control group (group A) and a modelling group (group B). Each group consisted of 12 rats. The study was carried out in accordance with the guiding principles for research involving animals and approved by the Shanghai University of Sports authorities for animal experimentation. At 3 days before the experiment, all rats were initially acclimated to a treadmill (DSPT-202 multi-channel running, Hangzhou, Duanshi) for 15 min and accustomed to locomotion in the treadmill.

Protocol
The experiment consisted of three stages: an injury intervention stage (stage 1) for modelling, a test stage (stage 2) for seeking TBs and studying SEA, and a morphological study stage (stage 3).

Stage 1
The rats in group B were anaesthetised with an injection of 3 ml/kg 10% chloral hydrate into the abdominal cavity, then fixed on the board of a home-made striking device (figure 1). The site of the proximal vastus medialis (VM) of the left hind limb was marked for all rats in both groups, but only rats in group B were hit at the marked position by a stick dropped from a height of 20 cm with a kinetic energy of 2.352 J (figure 1). The striking injury was performed once per week. On the second day, rats in group B ran on the treadmill for 90 min at a 16° downward angle and a speed of 16 m/min. The modelling rats were rested for the remaining days of the week. This modelling lasted for 8 weeks with the same procedure taking place each week. As a blank control, the rats in group A did not participate in being hit or treadmill running in stage 1.

Stage 2
During the 8-week recovery period, all rats, including group A, were anaesthetised in batches. The rats lay supine on an operational board with limb immobilisation. The VM of the left hind limb was surgically exposed with no skin or fascia covering it. Then, the muscle was palpated by an experienced doctor with his thumb or index finger, seeking a small, hard bundle-like bump. Any bumps found were marked with a stud and identified as a possible TB.

Subsequently, after 1 h of superficial anaesthesia, the rat’s ear was pinched rapidly with a pressure of 5 kg, which led to a reflex contraction in the VM. The MEA from the contraction of VM muscle was recorded at the site of the TB. The same procedures were also performed in doubtful TBs in group A (if a possible bump could be palpated in rats in group A, the myoelectrical test was applied). However, no bumps were palpated in most rats in group A; in these cases the centre of the muscle fibres in the marked position was selected for myoelectrical examination.

Stage 3
In accordance with the results of the above tests, a muscle biopsy was taken at the marked site to study the morphology of the MT rP. Two to three specimens were taken in two groups and fixed with formalin. Subsequently, the specimens were treated with a series of conventional procedures, namely embedding in paraffin wax, sectioning, slicing and staining with haematoxylin and eosin. On embedding, the direction of muscle fibres was marked for guidance of later sectioning. The sections were cut along transverse and longitudinal directions according to the marked direction of specimens. During the

Figure 1  Self-made striking device: a hitting stick freely drops to strike the site of the proximal vastus medialis femoris of the left hind limb of rat.
sectioning procedure the first six sections were discarded and the following six sections were mounted on slides, and the last sections were then also discarded. Thus, 12–18 sections/per animal were prepared for cross-sections and longitudinal sections. The morphology of muscle fibres in the slides was observed and evaluated using a digital microscopic imaging system (Olympus DP70×400, Japan).

Statistical analysis
During processing of MEA, the features of electrical signals were classified. Their shapes, amplitudes, durations and frequencies were measured. The morphologies of the muscle fibres (transverse and longitudinal) were observed and the diameters of muscle cells were measured in the transverse section of each slide in both groups. Statistical analysis was performed using SPSS V.16.0 (SPSS< Chicago, Illinois, USA). The mean and SD were calculated and a comparison was made between the two groups using an independent samples t test. The level of significance was defined as p<0.05.

RESULTS
Taut band (TB)
A total of 30 TBs with SEA were identified in group B, an average of 2.5 per animal (30/12); none were found in group A.

MEA and potentials
In group A, an explosive intensive MEA appeared with no contraction of muscle fibres while inserting the second electrode, lasting for 450–500 ms (figure 2A,a). Following it, relatively sparse and smaller amplitude electrical potentials appeared with a low frequency of roughly 30 Hz (mean) (figure 2A,b). This MEA decreased gradually and disappeared in 600–650 ms (figure 2A,c). The shapes of the potentials were biphasic, starting from baseline (upward) to negative deflection (figure 2C). The mean amplitude and duration of the potentials were 158.75±124.84 μV and 2.75±0.71 ms, respectively. Between potentials, small upward waves appeared with average amplitude and duration of 19.21±0.53 μV and 1.6±0.62 ms (figure 2C), respectively. Over time, all electrical activity vanished (figure 2A,c).

In group B, an explosive intensive MEA also appeared with a transient contraction of muscle fibres in the MTrPs lasting for 400–450 ms (figure 3A,a;C,a). Following that, there was a low-voltage MEA with average frequency 65 Hz lasting for more than 30 s (figure 3A,b;C,b). Continually, unequal lengths of silence and scattered electrical potentials occurred irregularly, in phases of 2 min. Two kinds of potential could be identified: type I and type II. Type I was a biphasic waveform beginning with a positive curve (downward deflection), whose average amplitude was 462.50±221.60 μV and duration 3.25±1.49 ms (figure 3B). Type II also started as a positive curve and swung fast to an upward deflection (figure 3D), with an average amplitude of 343.13±218.84 μV and duration of 2.63±0.52 ms. The amplitudes and durations between the two types were not significantly different. The most common potentials were Type I. Between two potentials some small waves could be seen, which were similar to those seen in normal muscle fibres, but they

Figure 2  Myoelectrical activities (MEA) of normal muscle fibre: the original MEA in 2 s (A) with insertion potentials (a), endplate potentials (b) and electrical silence (c); (B) is an expansion of (a) and (C) is a frame from (A) shown by the arrow.
were difficult to identify (figure 3B,C). The potential amplitudes and durations of these two types were significantly greater than that in normal controls \((p<0.01)\).

With external stimulation, a reflex contraction of muscle fibres and a withdrawal response of the animal’s hind limbs was observed in both groups; however, the EMG responses between the two groups showed obvious differences.

In group A, transient mixed phases of spikes in short periods appeared, accompanying the contraction. Moreover, the electrical activity occurred in alternating phases, with electrical potentials interspersed with silence (figure 4A). Spikes appeared as regional intensive potentials and individual potential, and could not be distinguished in some regions (figure 4B). The average amplitude and duration of the spikes were \(675\pm53.45\ \mu V\) and \(2.63\pm0.52\ \text{ms}\), respectively. The amplitude in group A was significantly the greatest among all the other amplitudes \((p<0.01)\), but no difference in duration was found.

In group B, serial SEA with polyphasic potentials occurred in MTrP in the VM, which looked like a comb shape with an average amplitude of \(45\pm4.63\ \mu V\) (figure 4C) and shorter duration of \(1.31\pm0.59\ \text{ms}\) (figure 4D). This electrical activity appeared repeatedly (figure 4E). Obviously, its amplitude and duration were the lowest and shortest among all the others \((p<0.01)\), respectively.

**Morphology in optical microscope**

After staining with haematoxylin and eosin dye, the muscle fibres in group A were seen to be almost identical in terms of size, polygon shape, density, regular alignment and gap size in cross-section (figure 5A). The average diameter of the fibres was \(27.84\pm6.61\ \mu m\). In the longitudinal section, they were regular and of uniform thickness (figure 5C).

In the muscle fibres of group B, the gathered circular or ellipse muscle fibres were of different sizes in cross-section (figure 5B) and the average diameter was \(41.84\pm6.82\ \mu m\), which was bigger than that in group A \((p<0.01)\). Continuous inflating tapering muscular fibres could be found in the longitudinal sections (figure 5D). Some short cracks appeared in the muscle fibres in group B and increased spaces between the muscle fibres were observed in transverse and longitudinal sections. The quantity of cell nuclei in group B was clearly smaller than that in group A. These deformed muscle fibres were deeply dyed and always aggregated together in all the sections.

**DISCUSSION**

The present study uses, for the first time, an animal model of MTrPs built up with a repeated injury to investigate the changes of electrophysiology and morphology at MTrPs. The results from the experimental group revealed obvious changes in MEA and histomorphology compared with that of the control group.

**Methodology of modelling**

Anatomically, the medial vastus muscle of rat is relatively large. It is therefore easy to hit with a striking device. The nerve endings of this muscle often

![Figure 3](http://aim.bmj.com/)

**Figure 3**  Myoelectrical activities (MEA) of trigger points: the original MEA in 2 s (A,C) with insertion potentials (a) and endplate potentials (b); (B) is taken from (A) section (b), and (D) is taken from (C) section (b) (arrow).
concentrate in two areas: one is along the femoral medial towards the inner half of patellar and the medial side of the knee, and upwardly inclines in dispersion to the anterolateral central part, and the other is at the medial part of patella and anterior part of the femoral medial condyle. These positions are similar to that of the human body, in which the referred pain of MT rPs of the VM is felt in the front of the patellar and anteromedial knee, and sometimes in the fascia of the patella. In a previous study, either single strike or eccentric exercise could not obtain definite and persistent MT rPs in the VM of rats. Therefore, it is necessary to use double enhanced injuries combining strikes to the VM with eccentric exercises to build up certain and persistent trigger points in the muscle.

Moreover, the kinetic energy of 2.352 J used in the strike can only cause skeletal muscle contusion with intact skin, which is confirmed by the previous studies. One of the studies had also shown that, with a repeated loading exercise to the finger, an acute MTrP occurred but vanished in 7 days due to the lack of continuous intervention. Considering repeated injuries as one of the causes for producing trigger points, a repeated intervention, lasting for 8 weeks, was applied in this study. Usually, a repeated contusion to muscle may cause a protective contraction of muscle fibres. A tension band in the VM can be induced. In 2 months of rest after the repeated intervention, trigger points are found. Therefore, it is necessary to repeat this modelling intervention eight times within 2 months. Using such a repeated blunt mechanical injury in this animal model, a high quality of MEA and abnormal morphological images was obtained.

According to the anatomy of rats, the VM of rats are large enough to be palpated with the finger of an experienced clinical specialist. A little bump can be easily recognised in the soft muscles without skin and fascia covered. But whether the bump is a real MT rP or not still requires verification by EMG with fine needle electrodes. Myoelectrical devices and fine needle electrodes are used for two purposes: one is to verify the site of the MT rP and the other is for recording EMGs. While performing EMG recordings, the first needle must be inserted and moved slowly, because the aim for this is only to elicit an LTR, not break MT rPs. In order to get the ideal recordings of MEA induced by a mechanical stimulation, it is necessary to put the test animal into a superficial anaesthetic status. Usually, the rats are in a superficial anaesthetic status within 1 h of anaesthetization when the chloral hydrate in the animal has been exhausted by half. At this time, one can gain a reflex contraction and good recordings of MEA by pinching the rat’s ear rapidly with approximately 5 kg pressure, generating a flexion withdrawal reaction. This reflex contraction is a protective action with the purpose of avoiding injury. This phenomenon was also observed in another study.
from Liu et al. They injected Freund’s adjuvant into the ankles of rats to cause arthritis, and an obvious withdrawal reaction was induced by an external stimulation when the rat was in superficial anaesthesia.

Features of MEA at myofascial trigger points

A transient intensive MEA with high voltage immediately appeared in the muscle fibres in the normal control group and the injury model group while the second fine needle electrode is being inserted, which the literature refers to as ‘insertion potential’. This is a reflex response to the stimulation of needle insertion because of the amounts of acetylcholine quickly released from presynaptic nerve terminals into the neuromuscular junction. Subsequently, the sodium channels in the muscle cell membrane are open, and depolarisation of the membrane quickly reaches the threshold to produce insertion potentials. Along with the insertion potentials, a few smaller potentials occur and gradually attenuate until completely disappearing in normal muscle fibres. The potentials are identified as normal endplate potentials by nerve electrophysiologists. The amplitude of these potentials is roughly 20 μV, which is identified as endplate noise. Different from that of normal control, the frequency of the endplate potentials in MTrPs is more intensive and the duration is longer, and two shapes of endplate potential can be identified. According to electrophysiologists, these two potentials were classified as two types of fibrillation potentials originating from the abnormal endplate, which were also SEA. These potentials disappear within a certain period, but appear again at an intermittent rate without any muscle contraction. This phenomenon had also been found by Hubbard and Berkoff when using a fine needle electrode. The SEA with a series of high peak discharges (>100 μV) and high frequency at the TB of trapezius occurred without any muscle contraction in human patients with an intermittent electrical silence. Repeating this study, using a sensitivity of over five times and increasing sweep speed, Simons et al. found

Figure 5 Microscope views of muscle fibres with haematoxylin and eosin staining, 400 × amplification: The upper panel shows a cross-section (A, B) and the lower panel shows a longitudinal section (C, D). The normal muscle fibres of group A appear the same size and polygonal shape, with regular alignment and gaps (A), and with well organized and uniform thickness (C). The abnormal muscle fibres of myofascial trigger points (MTrPs) show a gathering circular or elliptical shape with different sizes (B), and reveal continuous inflating and tapering fibres (D). The solid arrows indicate thick fibres and the open arrows indicate thin fibres. In (B) and (D), the abnormal fibres aggregate together, showing short cracks and large gaps between the muscle fibre framework.
two distinct components in these MEAs as well, which were the discontinuous variable high peak potentials and continuous low peak potentials. From the shapes of these two potentials, the one with high voltage was considered as the abnormal spontaneous endplate potential, and the other one with low voltage as endplate noise. However, these abnormal potentials resulted in two various interpretations. Some researchers have suggested they are intramuscular discharges, while others consider them to be abnormal discharges from the endplate in MTrPs. In the present study, we only considered them to be abnormal endplate discharges because they come from the MTrPs in the injury model.

Simons et al. proposed a ‘trigger point hypothesis’, in which one of the causes of abnormal potentials in MTrPs was due to leakage or an excessive release of acetylcholine in the presynaptic membrane of the neuromuscular junction. In the present study two abnormal potentials were also found, which is basically similar to the observations of Simons et al, and the amplitudes of the abnormal potentials are also higher than that of normal muscle fibre, thus supporting this hypothesis. However, it is difficult to explain why the myofascial trigger points have abnormal reverse endplate potentials if there is only a simple leakage or an excessive release of acetylcholine. Hong and Simons proposed that the MTrPs consisted of a sensory (nociceptive) component and a motor component that was in abnormal endplates with evidence of SEA. Therefore, some pathological changes in the cell membrane at the abnormal endplates possibly occur, such as changes to sodium and calcium ion channels.

While providing external stimulation in order to create a withdrawal response, the MTrPs produce a series of comb-like electrical activities with low voltage, different from the type I and II potentials. It seems that there is not enough acetylcholine to be released into the neuromuscular junctions from the presynapse due to exhausting acetylcholines in the nerve endings. Therefore, MEA recorded at MTrPs becomes weak. This phenomenon of MEA with external stimulation also supports the hypothesis of acetylcholine leakage. This MEA can be classified as pathological fibrillation potentials with less than 45 µV of amplitude. The duration of the comb-like potentials is longer than that of normal controls. One explanation is that the local involved muscles may still be in the stages of nerve damage or healing of nerve function. The other explanation for this condition is that the conduction velocity of nerve fibres and the contraction time of muscle fibres are different in the same motor unit. It may imply that the damage and recovery processes of nerve bundle and muscle fibres can be at different stages. According to the pathophysiology, these are mixed phase potentials and short wave multiphase potentials.

**Morphology of myofascial trigger points**

Following localisation of the TB by palpation, verified by EMG, MTrPs were localised and biopsied at the marked site of rat VM in vivo. Under optical microscopy enlargement (400 ×), the muscle cells appear large with small circular and/or elliptical shapes in the cross-section and enlarged tapering shapes in the longitudinal section in MTrPs, but the morphology of normal muscle shows a constant size, polygonal shape and orderly arrangement. In the early stage, Simons et al. also described muscle fibre of MTrPs with the features of large and small round fibres in cross-section. They hypothesised that the large one was the middle part of a contracture knot and the small one was the distal part of knot. According to the morphological features of MTrPs in cross-section, they made a hypothetical sketch of an enlarged tapering fibre in longitudinal section. In the present study, the appearance of muscle fibres in MTrP in the longitudinal section supports their deduction. Moreover, the diameters of those fibres are larger than the normal muscle fibres, similar to the measurements in a previous study from other researchers.

When a small amount of anticholinesterase (disopropylfluorophosphonate (DFP)) was injected into the proximal gastrocnemius muscle, disrupted fibres and vertical stripes with marked spasm in the injection site were observed, which was understood as increased acetylcholine in the endplate causing damage to muscle fibres, which may produce an abnormal spasm in local muscle fibres. The present study used optical microscopy to observe the muscle fibres of MTrPs, in which the contracture knots show greater density than the normal muscle fibres, and that most muscle fibres in MTrPs show cracks, compared with normal controls. All the observations seen in the present study could suggest that the MTrPs induced by a blunt injury are different from the MTrPs induced by the injection of anticholinesterase (DFP) and other chemical mediators. Therefore, no comparable reports can be found in the literature for this phenomenon, except the paper from Simons et al. The diameters of the muscle fibres are also significantly different between the experimental and the control groups. The muscle fibres with MTrPs are in pathological contraction and form gradually into contracture knots. There are still large gaps between the contracture knots, which may fill with a large number of biologically active substances. According to the hypotheses of Simons et al., a persistence of sarcomere contraction possibly increased the consumption of local energy and decreased local blood circulation. Local ischaemia hypoxia might stimulate the microvasculature to release some specific reactive substances, which could cause local pain near MTrPs, and these substances could also stimulate acetylcholine to be released abnormally and further contribute to continuous sarcomere contraction in the endplate, which creates...
the vicious circulation of energy crisis. The results of the present study support this hypothesis.

CONCLUSIONS
The present study demonstrates that the active MTrPs can be provoked by repeated blunt injury to the muscles of rats. The histomorphology of model MTrPs in rats verifies the hypothetical sketch of MTrPs from Simons et al.\textsuperscript{17} The histomorphological appearance of MTrPs is one of abnormal muscle fibres, which gather together with a group of large circular or/and elliptical shapes in cross-section and continuous infiltrating tapering fibres in longitudinal section. The MEA of MTrPs is a series of abnormal SEA events with high frequency and amplitude and a downward deflection at initial trace. However, with external stimulation, the myoelectrical signals appear as mixed phase and multiphase potentials with a short wavelength, which may be linked to the pathological potentials. It may imply an exhaustion of acetylcholine in the nerve endings in MTrPs. The animal model developed for the present experiment may provide evidence that the abnormal spontaneous endplate potentials and morphological changes are the result of chronic MTrPs.

**Summary points**

- A model for myofascial trigger points was created by blunt trauma and eccentric exercise in the left vastus medialis (VM) of 12 out of 24 SD rats.
- On average 2.5 taut bands (TB) were found in the left VM of model rats.
- Myoelectric activity and muscle morphology differed in the left VM of model rats compared with controls.

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