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SHORT COMMUNICATION

Histomorphological evidence of muscle tissue damage and recording area using coiled and straight intramuscular wire electrodes

Hiroyuki Tamaki · Fusayoshi Murata · Hiroaki Takekura

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Abstract While intramuscular wire electrodes (IWE) for the measurement of neuromuscular function offer high spatial resolution for examining single motor unit activity, the resulting damage to muscle tissue and mechanical instability should be considered. We examined the influence of IWE type and component parts on muscle damage using light microscopy in rats and confirmed that intramuscular pressure influences the mechanical stability of IWE. Three types of electrode, coiled electrodes with or without suture material inside and a straight electrode, were inserted into the soleus, gastrocnemius and tibialis anterior muscles. Transverse serial sections (5 μm) of these muscles in the vicinity of the electrodes were stained with haematoxylin and eosin. Less structural damage was observed in the vicinity of the recording points (leading-off surface; 50 μm diameter) for all electrode types compared to the electrode body. No differences in the extent of tissue damage were observed around the recording points for all electrodes. However, compared to straight electrodes, the extent of damaged tissue around the bodies of coiled electrodes was significantly ($P < 0.0001$) greater. The average distance between the recording points and the electrode body was <1 mm for all electrodes. Intdramuscular pressure at rest and maximal twitch contraction were 1.1 ± 0.5 and 49.4 ± 4.0 mmHg, respectively. Coiled IWEs became well integrated with muscle fibres, stabilizing electrode localization and facilitating electromyographic recordings without causing significant muscle damage.

Keywords Intramuscular wire electrode · Muscle · Electromyography · Histomorphometry · Coil

H. Tamaki (✉) · H. Takekura
Department of Physiological Sciences,
National Institute of Fitness and Sports, 1,
Shiromizu, Kanoya, Kagoshima 891-2393, Japan
e-mail: tamaki@nifs-k.ac.jp

F. Murata
Department of Anatomy, Faculty of Medicine,
Kagoshima University, Sakuragaoka,
Kagoshima 890-8520, Japan

Introduction

Electromyography (EMG) is a suitable method for studying neuromuscular disorders and their electrophysiological characteristics. However, while intramuscular EMG is an invasive procedure that uses fine wire electrodes to detect single motor unit activity with a high spatial resolution, it has better detection than surface EMG. Two general types of intramuscular wire electrode (IWE), straight and coiled, have been used in human and animal EMG studies (Caldwell and Reswick 1975; Prochazka and Davis 1992), with the more flexible coiled type usually being used for long-term recordings of motor unit activities to overcome problems associated with damage caused by exposure to the tensile and torsional forces acting on the wire during muscle contraction (Prochazka and Davis 1992). Two problems are associated with recording motor unit activity using IWEs. The first is the widespread muscle damage that results from the insertion of the needle with IWE, which in turn leads to limited detection of motor unit action potential. The second is that the mechanical instability of the inserted wire electrode results in unstable detection of motor unit action potential, i.e. significant variability in the waveform and amplitude. Several histological studies have shown no detectable effects on muscle performance after the removal of IWE (Mortimer 1981; Scheiner and Mortimer 1990). Observations of muscle tissues into which electrodes have been inserted previously enable us to identify the relative localization of IWE and the surrounding muscle fibres directly. However, sampling a large number of human muscle tissues to obtain the aforementioned data is difficult. In addition, data from animal muscle tissues obtained to date has illustrated the structural and functional merits and demerits of various types of IWEs, and thus what the appropriate IWE is for a particular application in human electrophysiological studies.

In this study, we clarified the histomorphological characteristics of the extent of the damage to muscle fibres surrounding the wire electrodes and estimated the mechanical stability of the electrodes within the muscle tissue.

Methods

Electrodes

Single copper wires (50 μm diameter) coated with polyurethane were twinned and used to prepare two types of electrode, coiled electrodes with or without suture material inside and straight electrodes. The coiled wire electrodes were made by carefully winding the twin wires around a mandrel made of 100-μm-diameter piano wire (Mortimer 1981; Prochazka and Davis 1992). Suture material made of silk was inserted inside the coil to facilitate identification of the area inside the coiled electrode ($n = 48$) once it was embedded in muscle. This method enabled us to clarify the difference between the area within, and the muscle fibre around, the coiled IWE in histological cross-section. Once fabricated, the coiled and straight electrodes were loaded into a hypodermic needle (24 gauge), leaving a small barb protruding from the needle cannula (distal 1.5 mm end of the needle; Fig. 1a). The coiled wire electrodes had a pitch of 257 ± 18 μm, an inside diameter of 184 ± 9 μm and an outside diameter of 284 ± 9 μm.

Histological procedure

Following anaesthesia of male Wistar rats ($n = 24$, age: 19.5 ± 4.4 weeks, weight: 324 ± 22 g), electrodes ($n = 144$) were inserted into the soleus (Sol), gastrocnemius (MG) and tibialis anterior (TA) muscles in the desired position, which was approximately parallel to the muscle fibres (Scheiner and Mortimer 1990). The

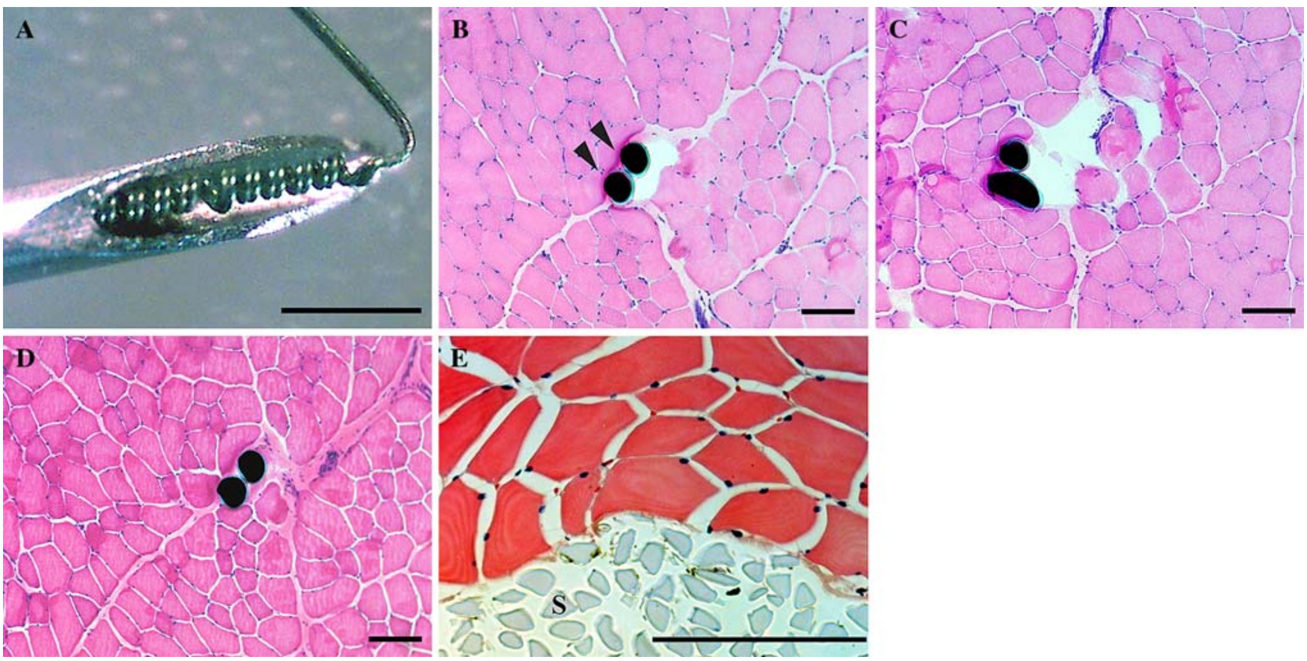

Fig. 1 Light micrographs of wire electrodes and cross-sections of muscle tissue with inserted electrodes. **a** Coiled wire electrode loaded into a hypodermic needle. **b** Extent of damage around the body of a straight wire electrode. **c** Extent of damage around the body of a coiled wire electrode. **d** Top of the hook of a coiled electrode (recording point). **e** Contact surface between muscle fibres and suture material (S). Cross-sections of the electrodes, which appear as two dark circular structures in **b**–**d** (*arrowheads*). Bar in **a**: 1 mm. Bar in **b**–**e**: 100 μm

muscles were removed and quickly frozen in isopentane, cooled using liquid nitrogen for carboxymethyl cellulose (CMC) gel block preparation or fixed with 3.5% glutaraldehyde in 100 mM sodium cacodylate buffer (pH 7.25 at 4°C) containing 1% sucrose for 2–3 h for paraffin block preparation. We used the method of Kawamoto and Shimizu (2000) for sectioning the frozen sample containing the hard material . The frozen muscle tissues were re-embedded in 5% CMC gel and frozen. Each CMC block was covered with a polyvinylidene chloride film coated with synthetic rubber cement (Cryoglue, Finetec Co, Ltd., Japan). Serial transverse sections (5 μm) with the film were cut using a cryostat (CM3050S, Leica, Germany) with a disposable tungsten carbide blade (TC-65, Leica) at −20°C. Each paraffin block was also cut into 5-μm sections using a microtome (Erma Inc., Japan). Specimens were stained with haematoxylin and eosin. All procedures using animals were carried out in accordance with the guidelines presented in the "Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences", published by the Physiological Society of Japan, and were approved by the Animal Care Committee of the National Institute of Fitness and Sports.

Morphological analysis

Quantitative analysis of histological observations was performed using a digital microscope (Coolscope, Nikon, Japan) and a CCD video image analysis system (Olympus, Japan), which comprised a light microscope (DX60, Olympus), a video monitor and a personal computer with image analysis software (Image-Pro Plus ver. 5.0, Media Cybernetics, MD, USA). The damaged areas in the transverse sections of the muscle tissues were defined as those areas showing conformational abnormalities or areas that were blank around the wire. The damaged areas (μm^2) and mean diameter (μm) were measured using image analysis software.

Intramuscular pressure

In order to examine the mechanical factors associated with the penetration of muscle fibres between the coils of the IWE that contribute to the mechanical stability of the electrode, intramuscular pressure (IMP) was measured at rest and at maximal twitch contraction evoked by sciatic nerve stimulation. A miniature transducer-tipped catheter (model SPR-477, 3F, Millar Micro-Tip, Houston, TX) was inserted at an angle of approximately 20° in line with the force-generation axis of the fibres and the thickest proximal portion of the TA ($n = 12$ subset of the total 24 rats). The catheter for IMP recordings was zero adjusted and calibrated with an electrical input before each experiment. The IMP signal was sampled at 1,000 Hz through an A/D converter (PowerLab 8SP, ADInstruments, Japan). Muscle contractions were evoked by electrical nerve stimulation (Takekura et al. 2003). Briefly, the rats were anaesthetized using sodium pentobarbitone (50 mg kg^{-1} body weight) and placed on a heated plate to maintain body temperature. The skin covering the buttocks was cut to expose the sciatic nerve and to separate it from the surrounding tissues. The sciatic nerve was then cut proximal to the stimulus electrode, which was connected to the stimulator (SEM-4201, Nihon Kohden, Japan) that employed supra maximal square-wave pulses of 0.1 ms in duration. The distal tendon of the TA was oriented along the natural pull of the muscle and attached to an isometric transducer (TB-654T, Nihon Kohden, Japan).

Statistics

Results are expressed as the mean ± standard deviation (SD). The significance of the differences between groups was calculated by analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test. Two-factor ANOVA was used to examine the individual effect on muscle damage among different electrode types and portion. The level of significance was set for P values less than 0.05.

Results

No significant difference was observed in the muscle damaged area around the recording points for all electrode types (straight type: 3541 ± 1,694 μm^2, coil without suture: 3,159 ± 2,377 μm^2, coiled with suture: 3,614 ± 2,606 μm^2; Fig. 1d). However, the damaged area around the electrode body was significantly ($P < 0.0001$) larger in the coiled electrode (50,738 ± 3,316 μm^2) than it was around the straight electrode (10,111 ± 1,571 μm^2; Fig. 1b, c). Two-factor ANOVA showed that the electrode type and the component parts had significant effects ($P < 0.0001$) on the area of muscle tissue damaged (Fig. 2). The average distance between the recording points and the damaged area around the electrode body was 1,017.2 ± 1,88.1 μm for all electrode types. For the coiled electrodes with sutures inside, the suture area (3,2890 ± 8,079 μm^2) accounted for approximately 65% of the damage in the area around the body of the electrode, which was not significantly different from the area inside the coiled electrode. The surface of the

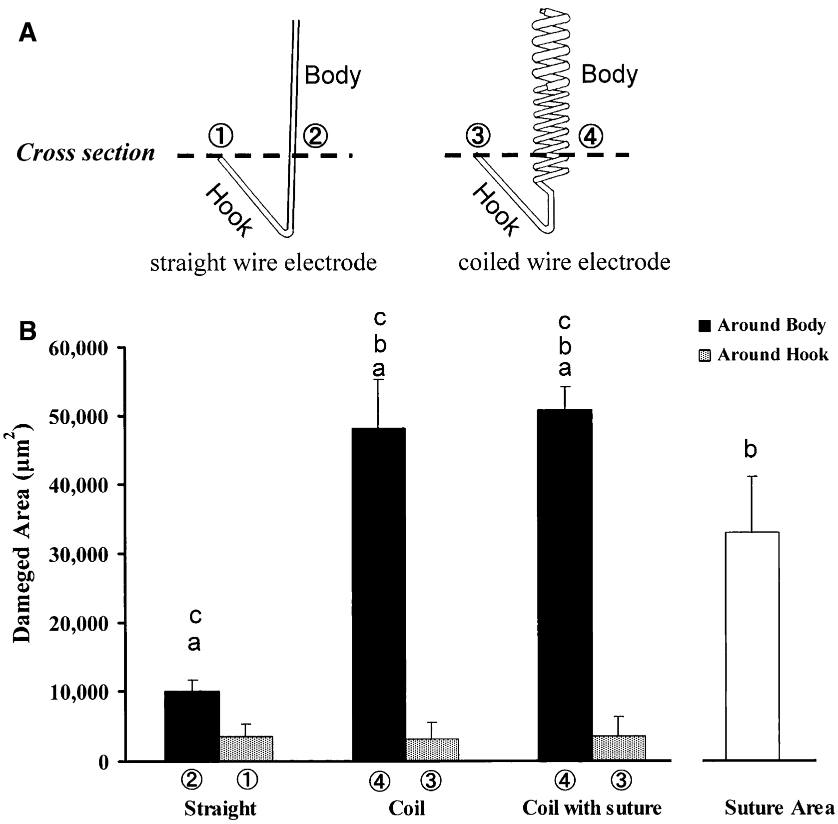

Fig. 2 A schematic diagram of the straight and coiled electrodes illustrating the difference between the recording point (*1*, *3*) and the electrode body (*2*, *4*) (**a**) and effects of electrode type and component part on the extent of muscle damage around the electrode body and the hook portion (**b**). [a]$P < 0.0001$ versus hook in each electrode, [b]$P < 0.0001$ versus body of straight electrode, [c]$P < 0.0001$ versus suture area

suture material on the opposite side of the wire (50 μm diameter) was in direct contact with the muscle fibres (Fig. 1e). The average distance between the edge of the suture and the muscle fibres was 14.8 ± 14.3 μm, and histological examination revealed that muscle fibres became well integrated within the pitch of the coiled electrodes.

Mean values for intramuscular pressure at rest and at maximal twitch contraction were 1.1 ± 0.5 and 49.4 ± 4.0 mmHg, respectively.

Discussion

Two major findings are reported here. The first is that insertion of the coiled electrodes resulted in greater muscle fibre damage around the electrode body, but that significant muscle damage was not observed around the recording points. This suggests that the magnitude of the damaged area is influenced by the shape and/or component part of the IWE. We assessed the histomorphometric characteristics of muscle tissue in the area from more than 1 mm distant of the recording points to the damaged area around the electrode body. All types of electrodes were associated with damage extending in radii of less than 40 μm around the recording points, suggesting that the straight and coiled wire electrodes were both capable of recording the action potentials of non-damaged muscle fibres at distances from 40 μm to 1 mm from the recording points. Given that the maximum electrical recording area for fine wire electrodes is, at most, within a radius of 300 μm (Gath and Stalberg 1982), the muscle damage attributed to needle insertion likely have a minor influence on EMG recordings.

Our second major finding was that muscle fibres became strongly associated with the coils of the electrode. This was evidenced by the fact that, in some cases, the fibres were in partial contact with the suture material inside the electrode. Moreover, the average distance between the muscle fibres and the suture material (14.8 μm) was significantly less than the 50-μm diameter of the wire, suggesting that muscle fibres would penetrate the spaces between the coils along the entire length of the electrode. Furthermore, higher intramuscular pressures would lead to increased penetration of muscle fibres between the coils of the electrode, and thus contribute to the mechanical stability of the IWE. Therefore, in considerations of intramuscular pressures at rest and during contractions in rats and humans (Sjøgaard et al. 1986), the use of coiled IWEs would ensure greater mechanical stability within muscle tissue, especially during static contractions in humans when muscle contractions increase intramuscular pressure at the expense of more muscle damage around the body of the electrode. These morphological findings of the relationships between various types of IWE and muscle fibres would thus be helpful for selecting the merits and demerits of specific electrodes for electrophysiological studies in humans.

The findings of this study revealed that (1) the muscle damage associated with the insertion of IWE did not extend around the recording points and (2) the presence of muscle fibres within the coil pitch contributed to the mechanical stability of coiled IWEs.

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