In Vivo Magnetic and Electric Recordings from Nerve Bundles and Single Motor Units in Mammalian Skeletal Muscle

Correlations with Muscle Force

F. L. H. GIELEN, R. N. FRIEDMAN, and J. P. WIKSWO, JR.

From the Living State Physics Group, Department of Physics and Astronomy, Vanderbilt University, Nashville, Tennessee 37235

ABSTRACT Recent advances in the technology of recording magnetic fields associated with electric current flow in biological tissues have provided a means of examining action currents that is more direct and possibly more accurate than conventional electrical recording. Magnetic recordings are relatively insensitive to muscle movement, and, because the recording probes are not directly connected to the tissue, distortions of the data due to changes in the electrochemical interface between the probes and the tissue are eliminated. In vivo magnetic recordings of action currents of rat common peroneal nerve and extensor digitorum longus (EDL) muscle were obtained by a new magnetic probe and amplifier system that operates within the physiological temperature range. The magnetically recorded waveforms were compared with those obtained simultaneously by conventional, extracellular recording techniques. We used the amplitude of EDL twitch force (an index of stimulus strength) generated in response to graded stimulation of the common peroneal nerve to enable us to compare the amplitudes of magnetically recorded nerve and muscle compound action currents (NCACs and MCACs, respectively) with the amplitudes of electrically recorded nerve compound action potentials (NCAPs). High, positive correlations to stimulus strength were found for NCACs ($r = 0.998$), MCACs ($r = 0.974$), and NCAPs ($r = 0.998$). We also computed the correlations of EDL single motor unit twitch force with magnetically recorded single motor unit compound action currents (SMUCACs) and electrically recorded single motor unit compound action potentials (SMUCAPs) obtained with both a ring electrode and a straight wire serving as a point electrode. Only the SMUCACs had a relatively strong positive correlation ($r = 0.768$) with EDL twitch force. Correlations for ring and wire electrode-recorded SMUCAPs were 0.565 and -0.366, respectively. This study adds a relatively direct examination of action currents to the characterization

Address reprint requests to Dr. John P. Wikswo, Jr., Department of Physics and Astronomy, Vanderbilt University, Box 1807 Station B, Nashville, TN 37235.

Dr. Gielen's permanent address is Department of Neurology, Erasmus University Rotterdam, 3000 DR Rotterdam, The Netherlands.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/91/11/1043/19 $2.00
Volume 98 November 1991 1043–1061

of the normal biophysical properties of peripheral nerve, muscle, and muscle single motor units.

INTRODUCTION

An action current flowing in an electrically excitable biological tissue produces a magnetic field that is uniquely determined by the current density distribution. Recent advances in magnetic recording technology have made it possible to examine the action currents by using miniature pick-up coils to sense and record their magnetic fields at physiological temperatures. Because the magnetic recording probes are not directly connected to the tissue, distortions of the data due to changes in the electrochemical interface between the probes and the tissue are eliminated. This provides a more direct characterization of action currents than has been attainable with conventional electrical recording. The present study examines the magnetically recorded action currents of mammalian fast-twitch skeletal muscle. For corroboration and preliminary comparison, the magnetically recorded waveforms are presented with simultaneously recorded data obtained by using conventional, extracellular electrode configurations. To distinguish the signals recorded magnetically from those recorded with electrodes, the magnetically recorded waveforms are designated action *currents,* while the electrically recorded signals are noted as action *potentials.* A comprehensive comparison of electric and magnetic signals from whole muscles must await further development of mathematical models similar to those used for studies of single muscle fibers (van Egeraat, Friedman, and Wikswo, 1990), single axons (Roth and Wikswo, 1985*b*), and nerve bundles (Wijesinghe, Gielen, and Wikswo, 1990*a, b;* Wijesinghe and Wikswo, 1990).

The properties of the extracellularly recorded compound action potentials of mammalian skeletal muscle are well established. There is evidence that magnetic recording should enhance the accuracy of our understanding of the currents in this tissue. The room temperature magnetic recording system was evaluated in earlier studies (Wikswo, 1982; Wikswo, Samson, and Giffard, 1983; Wikswo, Henry, Samson, and Giffard, 1985) using single cell, invertebrate preparations (Roth and Wikswo, 1985*b*; Gielen, Roth, and Wikswo, 1986*a*). For these preparations, the magnetic recording technique appears to have advantages over conventional electrical recording methods, particularly with respect to the quantitative accuracy and stability of the recorded waveforms (Roth and Wikswo, 1985*b*; Wijesinghe, 1988). Moreover, the absence of any electrical contact with the tissue during magnetic recording reduces the stimulus artifact to nonsignificant proportions. Magnetic recording also eliminates movement artifacts such as those generated by the disturbance of the electrochemical interface around electrical recording sensors. The fundamental principles of magnetic signals from biological sources have been summarized (Wikswo, 1990).

The room temperature magnetic probe has been used to record a variety of compound action signals in nerves of invertebrates (Roth and Wikswo, 1985*a*; Gielen, Roth, Wikswo, and Brink, 1986*b*; Wijesinghe, 1988), nonmammalian vertebrates (Wijesinghe, 1988), and mammalian vertebrates (Gielen et al., 1986*a*), and in vertebrate skeletal and cardiac muscle (Gielen et al., 1986*a*). Recent studies have demonstrated that this probe could be used effectively in an operating room environment to record a compound action current directly from the median nerve of

a patient undergoing surgery to correct a carpal tunnel syndrome disorder (Wikswo, Henry, Friedman, Kilroy, Wijesinghe, van Egeraat, and Milek, 1990*b*). We have developed mathematical models for analyzing and interpreting these signals (Barach, Roth, and Wikswo, 1985; Roth and Wikswo, 1985; Woosley, Roth, and Wikswo, 1985; Roth, Gielen, and Wikswo, 1987, 1988; Wijesinghe, 1988). In this study we applied magnetic recording methods and mathematical analysis techniques to examine action currents in a mammalian neuromuscular system. For this purpose, we selected as an experimental preparation the common peroneal nerve and extensor digitorum longus (EDL) muscle in the hind limb of the rat. This preparation has been used successfully in the past for a partial electrophysiological characterization of a mammalian neuromuscular system; however, the earlier studies were unable to assess action currents due to the limitations of electrical recording techniques (Griep, Gielen, Boom, Boon, Hoogstraten, Pool, and Wallinga-de Jonge, 1982).

These magnetic measurements of action currents in bundles of muscle fibers and single motor units in a mammalian nerve–muscle preparation constitute the first step to developing a bridge between basic, invasive experiments on single fibers (van Egeraat et al., 1990) and recent, noninvasive measurements of electrically evoked and voluntarily activated magnetomyogram (MMG) signals recorded from muscles of the human hand with a high-resolution Superconducting QUantum Interference Device (SQUID) magnetometer (Wikswo, Friedman, Kilroy, van Egeraat, and Buchanan, 1990*a*). Thus, the present study should enhance our understanding of the nature of currents flowing in active, electrically excitable mammalian tissues and facilitate the evaluation of the clinical potential of magnetic recording techniques.

METHODS

General Procedures and Experimental Instrument Configuration

The right extensor digitorum longus (EDL) muscles of 12-wk-old, male Wistar rats were used for our investigation. The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (nembutal). Approximately every half hour, additional nembutal was injected intraperitoneally to maintain an appropriate level of anesthesia. Body temperature was monitored rectally for the duration of the experiment. During dissection, body temperature was maintained at 37 ± 1°C by a heated dissecting table. Exposed tissues were bathed with rat Ringer's solution at 37 ± 1°C. The entire length of the EDL muscle was dissected free from surrounding muscles. Only the blood supply and the neural connections of the muscle were left intact. The sciatic nerve was cut proximal to the common peroneal branch to avoid any reflex contributions to the measured signals. The EDL tendons of origin and insertion were cut at the knee and proximal to the metatarsus, respectively. During the recording procedures, the rectally monitored temperature was maintained between 35 and 37°C by a heating platform that also supported the animal. The right hind limb of the rat was positioned in a temperature-controlled saline bath in such a way that the EDL, electrodes, and magnetic pick-up coil were immersed in the saline. The exposed tissues of the preparation that were not immersed were maintained in a constant flow of 37°C air saturated with water vapor. The configuration of the experimental apparatus is shown schematically in Fig. 1. The tendon of origin was connected to a flat clamp, stably attached to the apparatus frame. The distal part of the EDL was threaded through a wire-wound, ferrite core that was encapsulated in epoxy (Stycast 1266; Emerson and Cuming, Inc., Woburn, MA). This toroidal core served as the pick-up coil for recording the magnetic signals generated upon stimulation of the muscle (Gielen et al., 1986*a*). The insertion

tendons were tied with a short, stiff thread to an isometric force transducer (52-9503; Harvard Apparatus, South Natick, MA).

All experiments were carried out at the optimal twitch length of the whole muscle (Wallinga-de Jonge, Boom, Boon, Griep, and Lammeree, 1980). The whole muscle twitch was generated by means of supramaximal stimulation of the common peroneal nerve, which was immersed in a small saline pool surrounded by muscles in the thigh. Electric and magnetic recordings from this nerve were taken from the immersed segment.

Electric signals were acquired with a differential amplifier (Tektronix AM 502). The magnetic signals were acquired and amplified with a room temperature probe and amplifier system

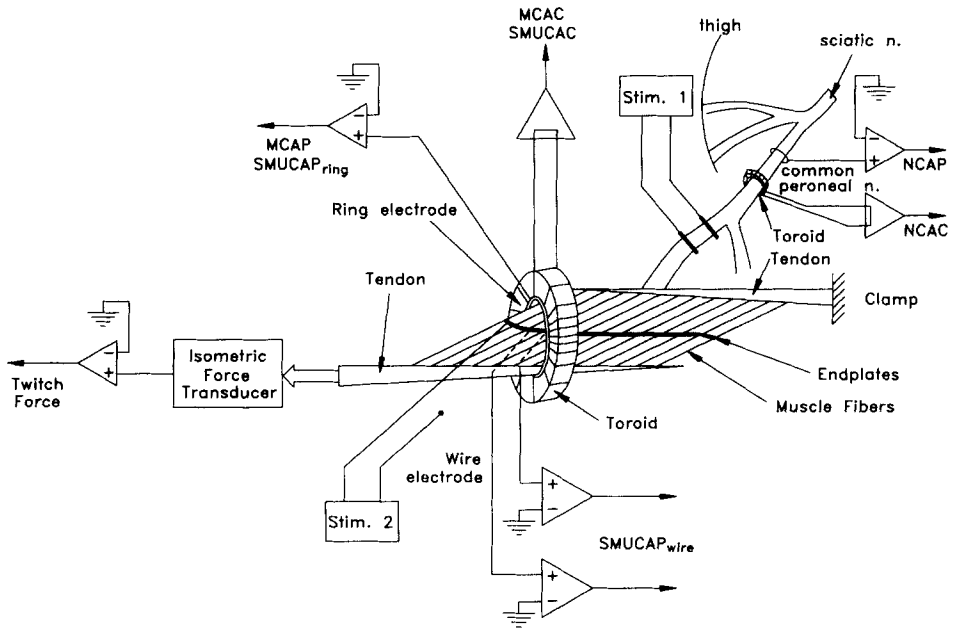

FIGURE 1. Experimental configuration for recording magnetic and electrical signals from the rat common peroneal nerve and EDL muscle. Two magnetic probes surround the common peroneal nerve and the EDL muscle. One records NCACs. The other senses MCACs and SMUCACs. Ring electrodes record extracellular NCAPs and muscle SMUCAPs. A wire electrode also records SMUCAPs. The origin tendon is fixed and force of evoked twitches is measured at the insertion tendon. Note that the sciatic nerve was cut to prevent reflex contamination of signals. The actual angle between the muscle fibers and tendons is ~12°. The magnetic probe and ring electrode are detailed in Fig. 2.

developed in our laboratory (Wikswo et al., 1983). Waveforms were monitored with a digital oscilloscope (Tektronix 5223) and digitized and processed with a signal averager (1170; Nicolet Instrument Corp., Madison, WI) for storage on 9-track magnetic tape. The data were analyzed using a microcomputer (model M380 [Olivetti Corp., Tarrytown, NY] with model 80386 processor [Intel Corp., Santa Clara, CA]). The analog bandwidth of the recording system was DC to 50 kHz (−3 dB) for the muscle force and for nerve and muscle compound action potential (N- and MCAP, respectively) signals, and 10 Hz to 30 kHz (−3 dB) for the nerve and muscle compound action currents (N- and MCACs, respectively). Either a single waveform or an average of 128 sequential waveforms was recorded. To accommodate the vast difference in duration of muscle twitch force and the NCAP, NCAC, MCAP, and MCAC signals, we adopted

the following sampling strategy: 1,024 sample points were acquired for every signal. The first 850 time samples were recorded with an intersample interval of 12 μs. The remaining 174 time samples were recorded with an intersample interval of 200 μs. In this way, the nerve and muscle compound action signals were sampled with a sufficiently high frequency, while the important features of the muscle twitch could be completely captured. Data acquisition for each signal was triggered by the electrical stimulus to the nerve. The interstimulus interval was 970 ms. The low stimulus rate was selected to avoid potentiation of the muscle force during the averaging procedure. We used standard linear regression analysis methods to obtain correlation coefficients for relationships of stimulus strength with magnetically and electrically recorded compound action signals.

Graded Stimulation of the Peroneal Nerve

The preparation described above offers an opportunity to obtain recordings of the magnetic fields arising from action currents in both the common peroneal nerve bundle and the muscle fibers that it innervates. To accomplish this, we applied electrical stimuli at levels ranging from just above threshold through supramaximal to the common peroneal nerve, which carries motor input signals to the EDL muscle. We recorded the magnetic compound action current signals from the peroneal nerve and the EDL, and the force of contraction (twitch) from the EDL. Because the twitch force of the EDL muscle serves as a reliable index of the degree of activity in the common peroneal nerve, we determined the correlation coefficients for the twitch force with the magnetically measured action current signals from both the nerve and muscle. Simultaneously, using conventional extracellular electrical recording techniques, we recorded muscle action potential signals and examined their correlations with the muscle twitch force.

Single Motor Unit Stimulation

In principle, it should be possible to use the magnetic and electric recording techniques described above to detect electrical activity at the single motor unit level. This requires stimulating a single axon and recording from a small, well-defined subset of muscle fibers. For the rat EDL muscle this means detecting a signal generated by 100 or fewer muscle fibers. The epimuscular microstimulation technique, described in detail elsewhere (Griep, Pool, Lammeree, Wallinga-de Jonge, Seeder, and Donselaar, 1980; Griep et al., 1982), was used to stimulate single axons in the common peroneal nerve and, thereby, single motor units in the EDL muscle. One of two 25-μm-diam stainless steel leads, insulated except at the tip, was manipulated in the end-plate region of the EDL until a stable stimulation of a single motor unit was obtained. The other lead was placed in the saline bathing the preparation and served to close the stimulation current loop. Criteria for stable stimulation (Griep et al., 1980) were satisfied if the single motor unit muscle twitch (SMUMT) amplitude and electromyogram (EMG) amplitude and shape showed typical all-or-none responses over a sufficient range of stimulus amplitudes. A pair of stimulus isolation units (850A; World Precision Instruments, Sarasota, FL) delivered a bipolar, rectangular stimulus pulse with durations of 30 and 20 μs for the positive and negative current pulses, respectively, so as to minimize the stimulus artifact in the electrical recordings. The amplitude of the negative pulse was adjusted to minimize the discharge duration of the capacitive component of the stimulating electrode impedance. In this way, the stimulus artifact duration could be limited in time to the total duration of the electronically generated stimulus pulse, i.e., ~50 μs. This procedure allowed us to record electrical signals close to the stimulating electrode without stimulus artifact interference.

Electric Recording of Nerve and Muscle Signals

The electric signals from the common peroneal nerve, the NCAPs, were recorded using a 125-μm-diam silver wire ring electrode. The electrode was chlorided to enhance recording

stability. The inner diameter of the ring (2.0 ± 0.3 mm) was just sufficient for enclosing the common peroneal nerve.

The electric signals of the EDL, termed the MCAPs, were recorded with two different types of recording electrodes, which, being "single fiber" and "surface" electrodes, simulated common electrode types used in clinical practice. We used 25-μm-diam stainless steel wires, insulated except at the tip, to simulate clinical single fiber EMG recordings. The wire electrodes were inserted into the muscle tissue ~2–4 mm in the longitudinal axis of the muscle belly. To reduce impedance, the tips of these electrodes were covered with an electrolytically deposited silver layer. At the end of each recording session, part of the silver from the tip was electrolytically deposited in the muscle to mark the recording position. Precise location of the recording site is essential for future histological studies. The relatively small dimensions of the

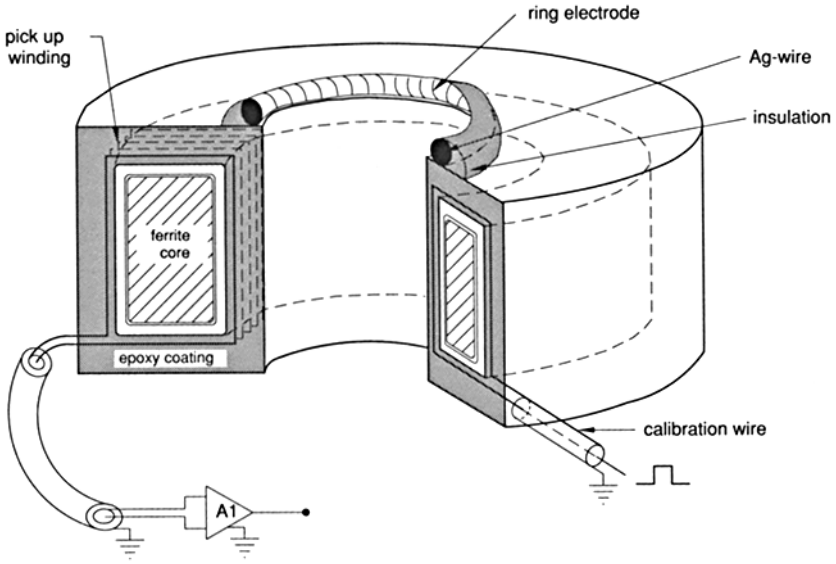

FIGURE 2. Schematic cross-section through toroid and ring electrodes. The toroid is composed of an epoxy-coated, wire-wound ferrite core. Dimensions of the epoxy-encapsulated devices used for nerve and muscle recordings are provided in the text. A Ag-wire ring electrode with inner radius equal to that of the coated toroid is attached to the toroid for electrical recording adjacent to the magnetic recording site. A turn of insulated copper wire (calibration wire) provides a calibration signal and permits monitoring of frequency compensation.

rat EDL, combined with our requirements for accurate histochemical analysis, prohibited our using conventional, coaxial needle electrodes. Previous studies indicated that these electrodes excessively damaged the EDL and prevented adequate processing of the tissue.

Surface electrode recordings were simulated with a 125-μm-diam chlorided silver wire ring electrode having the same inner diameter, 3.0 mm, as the toroidal magnetic sensor (toroid). The ring electrode was mounted on the face of the toroid (see Fig. 2), enabling recording of magnetic and electric muscle signals at nearly the same position on the muscle.

Magnetic Recording of Nerve and Muscle Compound Action Signals

The NCACs from the common peroneal nerve were recorded magnetically, using a toroidal pick-up coil. The toroid consisted of ~160 turns of 40-μm-diam insulated copper wire wound

on a ferrite core. The toroid (Fig. 2) had an inner diameter of 2.2 mm, an outer diameter of 4.8 mm, and a width of 2.5 mm. An independent, extra turn of the same diameter copper wire was used to calibrate and monitor frequency compensation for the magnetic recording system. The features of this system have been described in detail (Gielen et al., 1986*a*).

The magnetic signals from the EDL, termed MCACs, were recorded with a second, larger toroid, which consisted of 125 turns of 80-μm-diam copper wire wound on a ferrite core. The toroid had an inner diameter of 3.0 mm, an outer diameter of 5.9 mm, and a width of 1.6 mm. This toroid also had an independent calibration wire.

Motor Unit Type Characterization

Motor units in the rat EDL muscle are known to be of the FF (fast contracting, fast fatigue) and FR (fast contracting, fatigue resistant) types (Close, 1967, 1972; Kugelberg, 1973). By examining twitch fatigue characteristics, these types can be differentiated without histochemical analysis. While the two types of fibers have insignificant differences in their twitch shape and amplitude, their twitch responses to repeated 10-Hz stimulation differ markedly. After an initial increase due to potentiation, the SMUMT amplitude of the FF units decreases to ~10–20% of its initial value within 5 min. The FR units show no significant decrease in force within this period, unless their blood supply is compromised. At the end of this fatiguing stimulation/recording protocol, the electrode sites were electrolytically marked and the EDL muscle was fixed at the experimental length by a pair of rigidly connected clamps with separation adjusted to fit the proximal and distal tendons in situ. The muscle, with the attached wire electrodes, was then quickly removed from the animal and frozen in melting isopentane and subsequently in liquid nitrogen for later histochemical analysis of motor unit type and the positions of active motor unit fibers and recording electrodes.

RESULTS

Whole Muscle and Nerve Recordings

Compound action signals were evoked by 12 stimulus intensities ranging from just over threshold through supramaximal. Characteristic waveforms of resulting NCAPs, NCACs, and MCACs are shown in Fig. 3. Peak-to-peak compound action signal amplitudes for suprathreshold stimulation were: NCAP = 323 μV, NCAC = 0.411 μA, MCAC = 6.0 μA. Waveforms in this series of studies were produced by averaging the responses to 128 stimuli.

Interference between Nerve and Muscle Compound Action Signals

Fig. 4 shows typical recordings of nerve and muscle compound action signals that resulted from stimulating the intact peroneal nerve–EDL muscle preparation. The NCASs were much smaller than the EDL muscle–generated electric and magnetic signals that appeared only slightly later in time.

These muscle signals interfered with the NCAPs and NCACs even when the nerve signals were recorded at distances of several centimeters from the muscle. This interference is expected if one takes into account the greater diameter of the muscle cells (~50 μm) compared with that of the motor axons (~10 μm), and the fact that every motor axon of each FF-type motor unit in the rat EDL innervates 50–100 muscle fibers. This means that the action currents which the muscle cells inject into the surrounding medium are ~1,250–2,500 times greater than the NCACs, but only

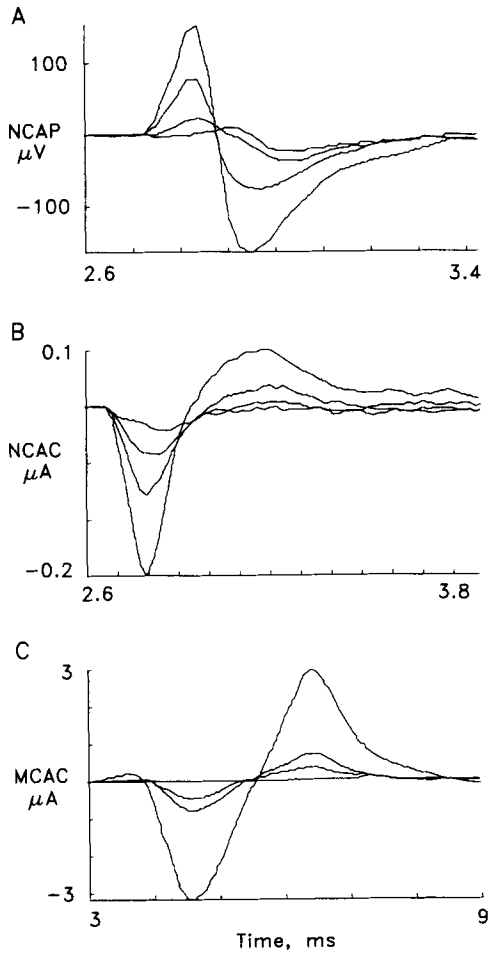

FIGURE 3. Effect of intensity of peroneal nerve stimulus on nerve and muscle compound action signals. *A* shows the electrically recorded NCAPs. NCACs (*B*) and MCACs (*C*) were recorded with magnetic probes. The waveforms shown are representative examples selected from responses to 12 stimulus intensities. All waveforms were produced by averaging 128 stimuli. Note the time base change (*C*) required to accommodate the greater duration of the MCAC.

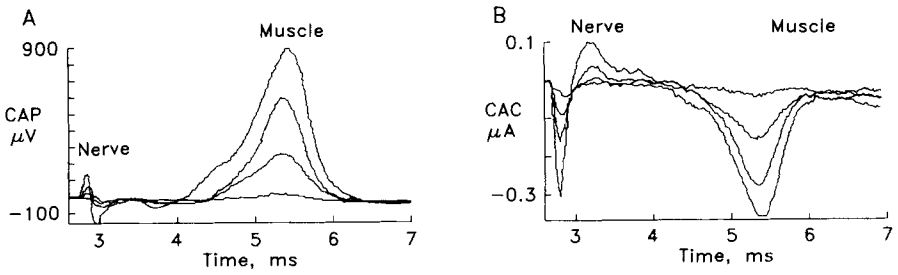

FIGURE 4. Muscle signal interference with NCAPs and NCACs. The NCAP (*A*) and NCAC (*B*) signals are interfered with by muscle action signals originating several centimeters distal to the nerve recording sites. Signals from nerve and muscle are indicated above the traces.

a small fraction of the muscle current will enter the toroid that is placed around the nerve. If the nerve has not been blocked to prevent its innervation of the muscle, the muscle signal can become an important limiting factor in either electric or magnetic NCAS recording, and care should be taken in interpreting these signals.

Because these studies comprise the initial magnetic examination of action currents in mammalian peripheral nerves and muscles, it was necessary to confirm that the

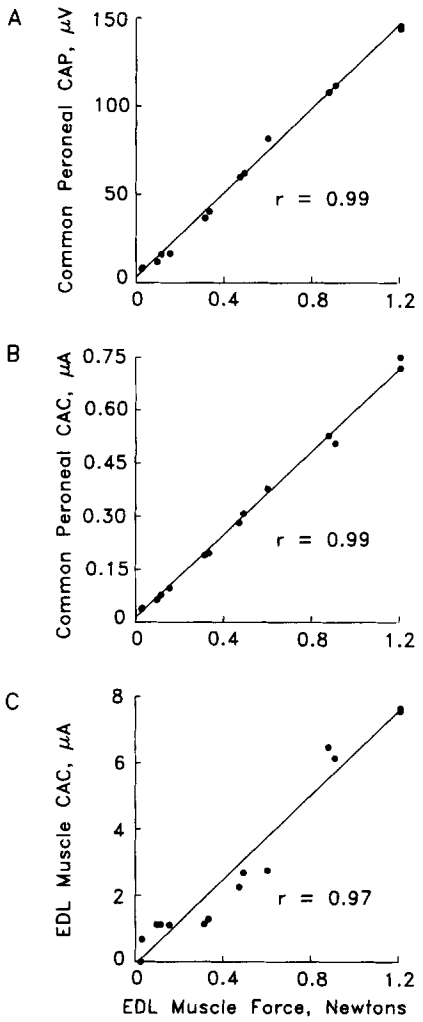

FIGURE 5. Correlations of electrically and magnetically recorded compound action signals with EDL muscle twitch force. Action signals were evoked by stimulation of the common peroneal nerve. Muscle length was set at optimal twitch length. Twitch forces were correlated with maximum peak-to-peak amplitudes of the nerve compound action potential (CAP) (*A*), and compound action current (CAC) (*B*), and the muscle CAC (*C*). Positive correlations (r) for all cases were significant ($P < 0.0001$). Force is indicated in newtons (= mass in kg × 9.8 m/s^2).

data were valid and quantitative representations of the electrical activity of these tissues, and to monitor the health of the preparation by established electrophysiological criteria. To this end, simultaneous magnetic and electric recordings were made of the electrically evoked activity of the rat peroneal nerve–EDL neuromuscular preparation. The contractile force generated by the muscle was also assessed. The respective sensitivities of the recording techniques were addressed by studying the

changes in each of the signals in response to variations in the strength of the stimulus applied to the nerve. Correlations between the amplitudes of the NCAP, NCAC, and MCAC with the peak muscle force evoked by graded peroneal nerve stimulation are shown in Fig. 5.

The data of Fig. 3 are subsets of the results summarized in Fig. 5, which shows the signal amplitudes of 12 recordings made at different stimulus intensities. As stated above, amplitudes of these signals are useful indicators of the activity in the nerve bundle or muscle.

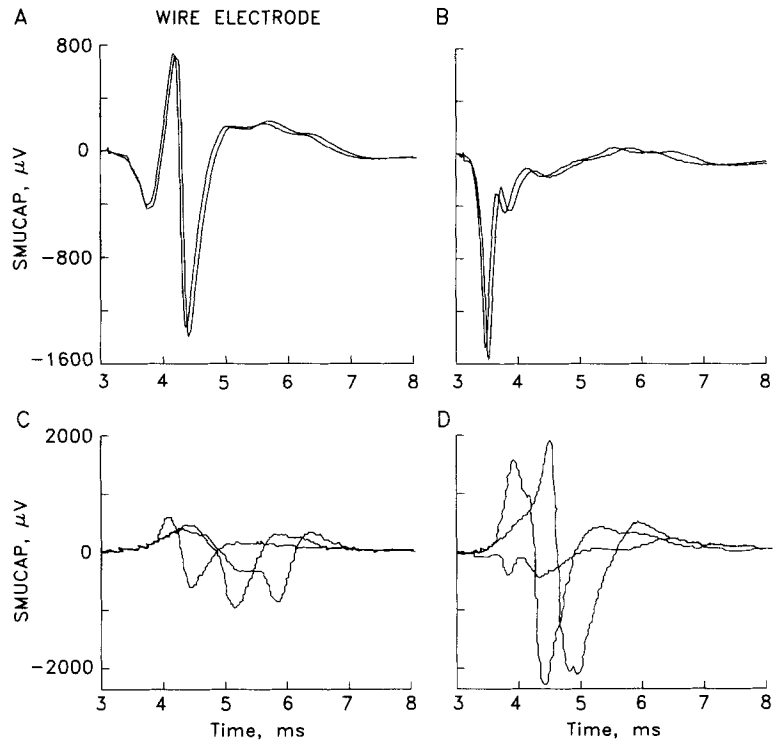

FIGURE 6. SMUCAPs recorded with single fiber type wire electrodes. (*A*) Variability of SMUCAP signals within one EDL muscle in which the same single motor unit is active during all recordings. Waveform examples were selected from recordings made with four electrodes placed at different positions within the motor unit territory. (*B–D*) Typical SMUCAP signals selected from a set of 40 electrode positions in 13 different EDL muscles.

High linear correlation coefficients (14 values per signal) of the data in Fig. 5, *A–C* ($r = 0.99$ for the NCAP, $r = 0.99$ for the NCAC, and $r = 0.97$ for the MCAC, respectively) relate the amplitudes of these waveforms to the muscle force. This confirms the established relationship of the NCAP with muscle contraction force and indicates that the magnetically recorded compound action currents have a similarly high degree of association with the level of activity in the nerve and muscle bundles.

Single Motor Unit Recordings

To further characterize the action currents generated in mammalian neuromuscular systems and to continue our parallel assessment of the validity of the magnetic recording techniques for studying neuromuscular electrophysiological events, we applied magnetic and electrical recording techniques to examine activity at single

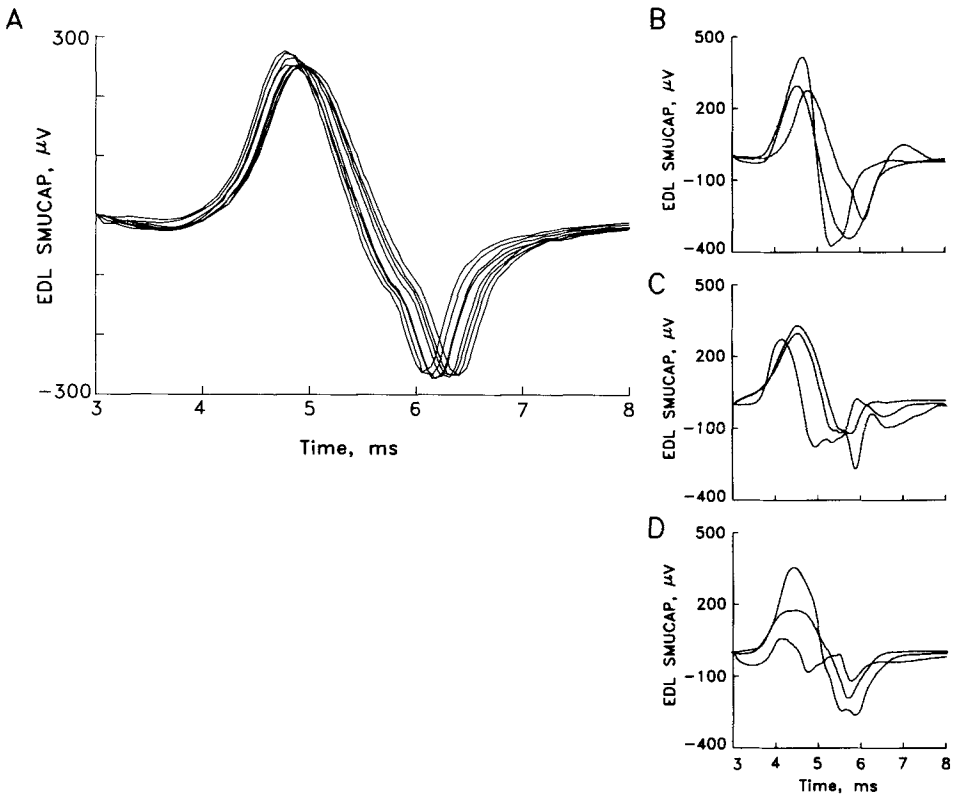

FIGURE 7. SMUCAPs recorded from the EDL muscle by a ring electrode around the muscle belly. This site is nearly identical to the SMUCAC recording position (see Fig. 8). (*A*) Typical variability of SMUCAP signals from one EDL muscle in which the same single motor unit is active during all recordings. Signals were recorded within a 2-h time span and became slower with the increasing duration of the experiment. (*B–D*) Typical signals representative of qualitatively similar waveforms recorded from nine different EDL muscles. Stimulus frequency was once per 970 ms.

motor units of the EDL. In this section, we present a summary of the results of experiments studying the electrically and magnetically recorded signals of FF-type single motor units of the EDL muscle.

Wire electrode recordings. Fig. 6 shows examples of single motor unit recordings, emphasizing the great variability in both shape and amplitude of the SMUCAPs, which makes these signals unique for a specific recording geometry (i.e., electrode

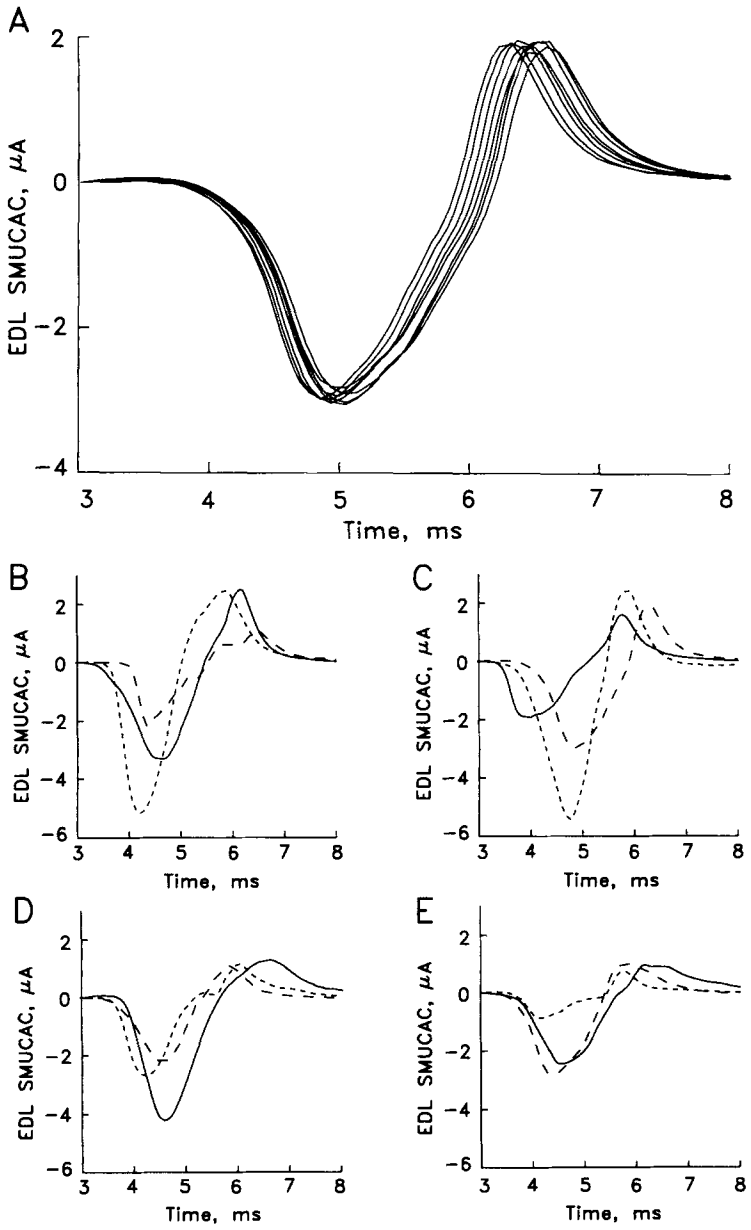

FIGURE 8. SMUCACs recorded by a toroidal probe around the muscle belly. This site is nearly identical to the SMUCAP recording position (see Fig. 7). (*A*) Typical variability of SMUCAC signals from one EDL muscle in which the same, single motor unit is active during all recordings. Signals were recorded within a 2-h time span and became slower with the increasing duration of the experiment. (*B–E*) Typical signals representative of qualitatively similar waveforms recorded from 13 different EDL muscles. Stimulus frequency was once per 970 ms.

placement). These signals are therefore suitable for quantitative tests of mathematical models that describe the generation of electric activity in muscles.

Surface electrode recordings. A surface electrode configured as a ring attached to the side of the toroid (see Fig. 2) was used to record the activity of all fibers of a motor unit. The shape of this type of electrode minimizes variations in signal

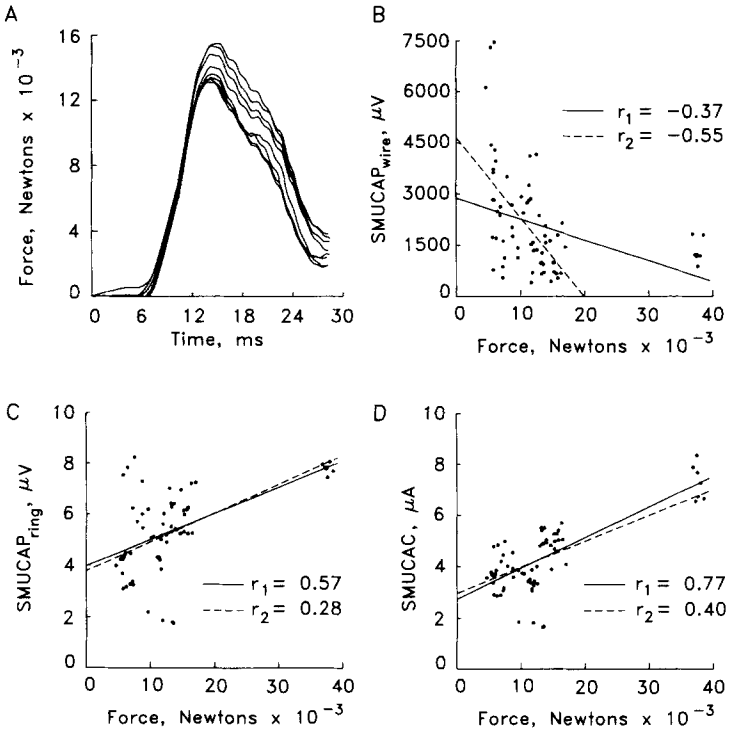

FIGURE 9. Correlations of amplitudes of electrically and magnetically recorded single motor unit compound action signals with EDL muscle twitch force amplitudes. Action signals were evoked by epimuscular microstimulation of a single endplate at the surface of the muscle belly. Muscle length was set for optimal force generation. (*A*) Typical twitch force responses to electrical stimulation of a single motor unit. 128 stimuli were applied with an interstimulus interval of 990 ms. Twitch forces were correlated with maximum peak-to-peak amplitudes of the single motor unit action signals recorded electrically by wire electrode (*B*), and ring electrode (*C*), and magnetically by toroidal probe (*D*). Due to clustering, at the high end of the force scale, of data points acquired early in these 2-h studies, correlations are shown for calculations including (r_1) and excluding (r_2) the high-force clusters (see Discussion). Data for correlation studies were obtained by 69 recordings from 9 EDL muscle preparations. Force is indicated in newtons × 10^{-3} (= mass in g × 9.8 m/s^2).

amplitude due to the distance between the recording electrode and the active fibers. The worst-case amplitude difference of a factor of two would be expected from two identical fibers, one in the center of the bundle, and therefore at the center of the recording electrode, and the other immediately adjacent the ring (Wijesinghe, 1988). Even so, the ring electrode is more uniform in its sensitivity to all fibers in a bundle

than are other electrodes; hence, the signal amplitude for a ring electrode should provide the best possible electrical measure of the total number of active fibers in a motor unit. Fig. 7 shows examples of SMUCAPs recorded with the ring electrode.

Magnetic recordings. Fig. 8 shows the results of magnetic recordings made of an EDL single motor unit under the same experimental conditions as used for the electrical recordings shown in Figs. 6 and 7. As expected from the spatial extent of a typical skeletal muscle fiber action potential and the spatial resolution of the toroid (Gielen et al., 1986*a*), the SMUCACs and surface electrode–recorded SMUCAPs are comparable in duration. Further quantitative studies based on volume conduction models should ultimately demonstrate the specific advantages and limitations of the two recording techniques.

Correlations of Single Motor Unit Action Signals with Muscle Twitch Force

The correlations between the amplitudes of the SMUMT force and ring and wire electrode–recorded SMUCAP and magnetically recorded SMUCAC amplitudes are shown in Fig. 9. 69 recordings were taken from nine EDL muscles in which force, electrical, and magnetic measurements were made simultaneously. For each muscle, all recordings were completed within a 2-h period. Relating the amplitude of the twitch force to the amplitude of the electrically and magnetically recorded compound action signals produced the following correlation coefficients: wire electrode–recorded SMUCAPs, $r_1 = -0.37$, $r_2 = -0.55$; ring electrode–recorded SMUCAPs, $r_1 = 0.57$, $r_2 = 0.28$; toroid-recorded (magnetic) SMUCACs, $r_1 = 0.77$, $r_2 = 0.40$. The rationale for calculating pairs of correlation coefficients is presented below (see Discussion).

DISCUSSION

Graded Stimulation Experiments

During graded stimulation of the peroneal nerve, activity in nerve and muscle is expected to be distributed more or less homogeneously over the cross-section of the tissue. This means that geometrical parameters, such as the positions of active fibers with respect to the recording sensor, are less dominant and limiting for the studies of nerve and muscle compound action signals than for single motor unit recordings. In the graded stimulation experiments, the lowest stimulus intensity used was selected to activate at least several motor units. With this paradigm, it was highly probable that active muscle fibers would pass through the toroidal magnetic probe and the ring electrode. As a result, the correlations of both the magnetically and electrically recorded NCASs and MCASs with muscle twitch force are high (see Fig. 5). This suggests that, as indices of activity in mammalian nerve/muscle systems, the magnetically recorded signals from both the nerve and muscle may be as valid as the electrically recorded signals.

Recordings from Single Motor Units

In this type of experiment, only a small, specific part of the nerve or muscle was activated. In the nerve, only a single axon was stimulated. The present study

demonstrates that the room temperature magnetic recording technique affords the degree of sensitivity necessary to examine motor unit signals resulting from the stimulation of single motor axons in intact, mammalian nerve bundles such as the rat common peroneal nerve (Gielen and Wikswo, 1985).

In the muscle, only a very limited number of fibers are activated during single motor unit stimulation. The fibers belonging to a motor unit are located in the motor unit territory, a restricted area of the cross-section of the muscle (Buchthal, Guld, and Rosenfalck, 1957; Griep et al., 1982). This means that, in the case of the EDL muscle, it is very likely that not all of the fibers in a particular motor unit will pass the toroidal pick-up coil or ring electrode (i.e., will pass through a plane that contains the recording sensor and is perpendicular to the longitudinal axis of the muscle). In such a case, it is not likely that the amplitudes of the MCASs will be proportional to the number of active motor unit fibers or the size of the motor unit. This is the most probable cause of the low values for the correlations of electrically recorded SMUCAPs with EDL twitch force shown in Fig. 9. Note that the actual angle between the muscle fibers and tendons is $\sim 12°$; therefore, the assumption that the toroid is perpendicular to the fibers will induce a minimal error, as cosine $12° = 0.978$. Another factor that may contribute significantly to the low correlations is the fact that muscle action potential characteristics change when the depolarization runs into the tendon and stops propagating (Griep et al., 1982; Heringa and Stegeman, 1987; Kleinpenning, Gootzen, Stegeman, and van Oosterom, 1990). A precise understanding of the relationship between the action signals from single fibers and the compound action signals from various recording sensors must evolve from future, thorough histological evaluations of the geometry of the recorded units with respect to the position of the probes.

It is possible to use the epimuscular stimulation technique employed in this study to activate other motor unit types (Griep et al., 1980); however, it is much more time consuming to meet the criteria for stable stimulation of motor unit classes other than the FF-type. This is probably due to a slightly lower stimulation threshold for the FF-type motor unit. This fact, combined with the mixed packing of muscle fiber types and the close spacing of the endplates in the rat EDL, impedes consistent epimuscular stimulation of units other than the FF-type. However, an exhaustive study of signals recorded from other motor unit types is not essential to the present, initial characterization of the relationship of magnetically measured action signals to nerve and muscle activity.

Single fiber type recordings. This type of recording is unique due to the very strong dependence on the distance between the active fibers and the electrode within the uptake area of the single fiber type of electrode (Stalberg and Trontelj, 1979; Griep et al., 1982). The spatial dependence is the most important reason why this type of signal can show widely varying MCAP shapes and amplitudes, as can be seen clearly in Fig. 6. The high spatial resolution and the relatively small uptake area of these electrodes are considered the most important features that determine the type of information that can be extracted from these recordings. This signal type is interesting for quantitative evaluations of volume conduction models, which are in many cases the bases for advanced signal analyzing programs (Griep et al., 1982;

Wijesinghe, 1988). The single fiber type recordings have found important clinical applications (Stalberg, Ekstedt, and Broman, 1971; Stalberg and Trontelj, 1979).

Surface electrode recordings. Potentials recorded via surface electrodes show a typical widening in time due to the spatial averaging effect of the electrode (Griep, Boon, and Stegeman, 1978). Here the signals lose detail as compared with the single fiber type electrode recordings, as the surface electrodes have a larger uptake area and less spatial resolution. The surface electrode technique enables the clinically interesting, totally noninvasive recording of physiological data. The data analysis of surface electrode signals often makes use of much less deterministic approaches, depending on the information of interest. In most applications, the analysis also relies heavily on the contributions of single fibers and the superposition principle. Therefore, the surface electrode signals can be treated as a special case of the single fiber type recordings, but the physiological information that can be extracted from each is clearly different.

Correlation of single motor unit action currents with contractile force. A deterioration of the force of contraction of single motor units is evident from the data in Fig. 9. This may be accounted for by physical and metabolic changes in the preparation during the 2-h duration of the experiments, during which the EDL generates 7,200 contractions. Enhancing the deleterious effects of normal fatigue on contractile force is the surgical interruption of distal circulatory elements that is unavoidable with this preparation. Also, the optimum twitch length was set once at the beginning of the experiment and not readjusted. Some changes in the elastic elements may occur during the 2-h period. In addition, histological studies show that, even with attention to moistening the preparation during the experiments, muscle fibers at the air-exposed surfaces of the preparation tend to swell. This may adversely affect their contractile properties. The reduction in contractile force over the duration of the experiments was observed regardless of the method of recording. Data from earlier trials in these experiments tended to form clusters at the high end of the contractile force scale (see Fig. 9, *B–D*). In consideration of the likely time-related changes in the preparation, coefficients relating the single motor unit action potentials and currents to the force of contraction were calculated with (r_1) and without (r_2) the clusters of early data points.

The amount of scatter showed variation with the recording techniques, with the magnetically recorded data showing the highest positive correlations (Fig. 9, *B–D*). Histological examinations comparing the viability of preparations used in submerged recording (magnetic) versus recording with the tissue moistened but in air (electrical) are necessary; however, speculation that the differences in scatter may be at least partially accounted for by submerged tissue effects, reduced movement artifacts and effects of tissue–electrode interface alterations would be consistent with earlier observations in this study.

Shape and Duration of Signals

The contribution from muscle signals shown in Fig. 4 is monophasic, with a duration nearly one-half that of the MCAC shown in Fig. 3 *C*. The onset time of the monophasic components in Fig. 4, *A* and *B* ($t \approx 4$ ms) and the maxima of the same signals ($t \approx 5.5$ ms) correspond with the onset ($t \approx 4$ ms) and main zero crossing (~ 5.5 ms), respectively, of the MCAC shown in Fig. 3 *C*. If we do not take the

duration of the signals into account, the MCAC in Fig. 3 *C* appears similar to the first derivative of the monophasic components in Fig. 4, *A* and *B*. The relatively large size of the toroidal sensor used for recording the MCACs (Fig. 3 *C*), compared with the spatial extent of the muscle fiber action potential recorded intracellularly along the longitudinal axis of the muscle, accounts for a reduction in spatial resolution which causes a widening of the signals (Gielen et al., 1986*a*). This may account for the apparent discrepancy in signal duration mentioned above. The similarity in shape and time envelope of the monophasic components in Fig. 4, *A* and *B*, is striking. It is also remarkable that the peaks of these monophasic components do not shift in time with increasing stimulus intensity, while the shifting clearly occurs in Fig. 3, *A* and *B*. It is not clear what causes these monophasic signals. The signals must be due to current flowing through the toroid as a result of the extracellular currents from the muscle. Most aspects of these signals conform with the qualifications given for nonpropagating signal components recently identified as far-field signals (Stegeman, van Oosterom, and Colon, 1987*a*; Stegeman, van Oosterom, and Notermans, 1987*b*). It may be reasonably concluded that these monophasic components cannot be due to movement artifacts. The delay between muscle excitation and mechanical contraction is well established. From Fig. 8, and from our whole-muscle twitch force studies which show that mechanical events commence only after a delay of $\sim$6 ms in this preparation, it is clear that mechanical effects occur well after the associated electric and magnetic signals. The duration of the whole-muscle mechanical force generation in the rat EDL was greater than that of the monophasic signals.

The magnetic probe system allowed us to examine biophysical properties of compound action currents in peripheral nerve, muscle, and muscle single motor units. This characterization of action current properties establishes a baseline of normal physiological data for fast-twitch skeletal muscle and the common peroneal nerve of the rat. Correlations of magnetically recorded compound action signals with muscle twitch force are similar to the values relating extracellular, electrically recorded compound action potentials to twitch force. The quality of the magnetic recordings of the nerve and muscle action signals was comparable to that of the electrical recordings. Inherent advantages of the magnetic technique, such as its insensitivity to movement artifacts and its relative stability with respect to variations in the interface of the recording probe with the tissue, make this technique particularly suitable for recording from neuromuscular systems. Furthermore, combining the magnetic measurements of action currents with intracellular recordings of membrane potentials should provide an accurate means of determining intracellular resistivities (Roth and Wikswo, 1985*a*, 1989). A limitation of the magnetic recording system used in these experiments is the necessity of threading the tissue through the toroid. While this did not hamper the present, acute experiments, the necessity of cutting a tendon or nerve would complicate or prohibit the use of the toroid for chronic studies. A variation of the technique employed in human nerve studies used a split toroid that could be opened, positioned around the nerve, and then closed (Wikswo et al., 1990*b*). Further development of the probe will be necessary to permit chronic studies of untraumatized tissue; however, the description of SMUACs presented in this paper will have a crucial role in the interpretation of noninvasive studies of single motor unit function that are now possible due to the development of high-resolution SQUID magnetometers (Wikswo et al., 1990*a*).

We thank Licheng Li for her skill and care in preparing the illustrations.

The research reported in this manuscript was supported by National Institutes of Health grants NS-24751 and NS-19794.

Original version received 26 October 1989 and accepted version received 17 June 1991.

REFERENCES

Barach, J. P., B. J. Roth, and J. P. Wikswo, Jr. 1985. Magnetic measurements of action currents in a single nerve axon: a core-conductor model. *IEEE Transactions on Biomedical Engineering.* BME-32: 136–140.

Buchthal, F., C. Guld, and P. Rosenfalck. 1957. Multielectrode study of the territory of a motor unit. *Acta Physiologica Scandinavia.* 39:83–104.

Close, R. 1967. Properties of motor units in fast and slow skeletal muscle of the rat. *Journal of Physiology.* 193:45–55.

Close, R. I. 1972. Dynamic properties of mammalian skeletal muscle. *Physiological Reviews.* 52:129–197.

Gielen, F. L. H., B. J. Roth, and J. P. Wikswo, Jr. 1986*a*. Capabilities of a toroid-amplifier system for magnetic measurements of current in biological tissue. *IEEE Transactions on Biomedical Engineering.* BME-33:910–921.

Gielen, F. L. H., B. J. Roth, and J. P. Wikswo, Jr., and P. R. Brink. 1986*b*. Action current propagation across an electrical synapse. Magnetic measurements on a septated earthworm axon. *Biophysical Journal.* 49:340*a*. (Abstr.)

Gielen, F. L. H., and J. P. Wikswo, Jr. 1985. Electric and magnetic motor unit action signals in the rat. *Proceedings of the 38th Annual Conference on Engineering in Medicine and Biology.* 27:340.

Griep, P. A. M., F. L. H. Gielen, H. B. K. Boom, K. L. Boon, L. L. W. Hoogstraten, C. W. Pool, and W. Wallinga-de Jonge. 1982. Calculation and registration of the same motor unit action potential. *Electroencephalography and Clinical Neurophysiology.* 53:388–404.

Griep, P. A. M., C. W. Pool, G. C. Lammeree, W. Wallinga-de Jonge, T. Seeder, and Y. C. Donselaar. 1980. Intramuscular and epimuscular microstimulation of single motor units. *Neuroscience Letters.* 17:191–196.

Griep, P. A. M., K. L. Boon, and D. F. Stegeman. 1978. A study of the motor unit action potential by means of computer simulation. *Biological Cybernetics.* 30:221–230.

Heringa, A., and D. F. Stegeman. 1987. Comments on "potentials produced by arbitrary current sources in an infinite- and finite length circular conduction cylinder." *IEEE Transactions on Biomedical Engineering.* BME-34:73–74.

Kleinpenning, P. H., T. H. J. M. Gootzen, D. F. Stegman, and A. van Oosterom. 1990. The equivalent source description representing the extinction of an action potential at a muscle fiber ending. *Mathematical Bioscience.* 101:41–61.

Kugelberg, E. 1973. Properties of rat hind-limb motor units. *In* New Developments in Electromyography and Clinical Neurophysiology. J. E. Desmedt, editor. Karager, Basel. 2–13.

Roth, B. J., F. L. H. Gielen, and J. P. Wikswo, Jr. 1987. A comparison of two modes for calculating the electrical potential in skeletal muscle. *Annals of Biomedical Engineering.* 15:591–602.

Roth, B. J., F. L. H. Gielen, and J. P. Wikswo, Jr. 1988. Spatial and temporal frequency dependent conductivities in volume conduction calculations of skeletal muscle. *Mathematical Bioscience.* 88:159–189.

Roth, B. J., and J. P. Wikswo, Jr. 1985*a*. The electrical potential and magnetic field of an axon in a nerve bundle. *Mathematical Bioscience.* 76:37–57.

Roth, B. J., and J. P. Wikswo, Jr. 1985*b*. The magnetic field of a single axon. A comparison of theory and experiment. *Biophysical Journal.* 48:93–109.

Roth, B. J., and J. P. Wikswo, Jr. 1989. Longitudinal resistance in cardiac muscle and its effect on propagation. *In* Cell Interactions and Gap Junctions. N. Sperelakis and W. C. Cole, editors. CRC Press, Boca Raton, FL. 165–178.

Stalberg, E., J. Ekstedt, and A. Broman. 1971. The electromyographic jitter in normal human muscles. *Electroencephalography and Clinical Neurophysiology.* 31:429–338.

Stalberg, E., and J. V. Trontelj. 1979. Single Fiber Electromyography. Mirvalle Press Limited, Old Woking, Surrey, UK.

Stegeman, D. F., A. van Oosterom, and E. J. Colon. 1987*a*. Far-field evoked potential components induced by a propagating generator: computational evidence. *Electroencephalography and Clinical Neurophysiology.* 67:176–187.

Stegeman, D. F., van Oosterom, and S. L. H. Notermans. 1987*b*. Theoretical aspects of far-field somatosensory evoked potential generation. *In* Evoked Potentials III. C. Barber and T. Blum, editors. Butterworth Publishers, Stoneham, MA. 261–264.

van Egeraat, J. M., R. N. Friedman, and J. P. Wikswo, Jr. 1990. The magnetic field of a single muscle fiber. First measurements and a core conductor model. *Biophysical Journal.* 57:663–667.

Wallinga-de Jonge, W., H. B. K. Boom, K. L. Boon, P. A. M. Griep, and G. C. Lammeree. 1980. *American Journal of Physiology.* 239:C98–C104.

Wijesinghe, R. S. 1988. Comparison of Electric and Magnetic Techniques for the Determination of Conduction Velocity Distributions of Nerve Bundles. Ph.D. Dissertation. Vanderbilt University, Nashville, TN. 311 pp.

Wijesinghe, R. S., F. L. H. Gielen, and J. P. Wikswo, Jr. 1990*a*. A model for compound action potentials and currents in a nerve bundle. I: The forward calculation. *Annals of Biomedical Engineering.* In press.

Wijesinghe, R. S., F. L. H. Gielen, and J. P. Wikswo, Jr. 1990*b*. A model for compound action potentials and currents in a nerve bundle. III: A comparison of electric and magnetic techniques. *Annals of Biomedical Engineering.* In press.

Wijesinghe, R. S., and J. P. Wikswo, Jr. 1990. A model for compound action potentials and currents in a nerve bundle. II: A sensitivity analysis of model parameters for the forward and inverse calculations. *Annals of Biomedical Engineering.* In press.

Wikswo, J. P., Jr. 1982. Improved instrumentation for measuring the magnetic field of cellular action currents. *Review of Scientific Instrumentation.* 53:1846–1850.

Wikswo, J. P. 1990. Biomagnetic sources and their models. *Advances in Biomagnetism.* S. J. Williamson, editor. Plenum Publishing Corp., New York. 1–18.

Wikswo, J. P., Jr., R. N. Friedman, A. W. Kilroy, J. M. van Egeraat, and D. S. Buchanan. 1990*a*. Preliminary measurements with microsquid. *In* Advances in Biomagnetism. S. J. Williamson, editor. Plenum Publishing Corp., New York. 681–684.

Wikswo, J. P., Jr., W. P. Henry, R. N. Friedman, A. W. Kilroy, R. S. Wijesinghe, J. M. van Egeraat, and M. A. Milek. 1990*b*. Interoperative recording of the magnetic field of a human nerve. *In* Advances in Biomagnetism. S. J. Williamson, editor. Plenum Publishing Corp., New York. 137–140.

Wikswo, J. P., Jr., W. P. Henry, P. C. Samson, and R. P. Griffard. 1985. A current probe for measuring cellular action currents. *In* Biomagnetism: Applications and Theory. H. Weinberg, G. Stroink, and K. Katila, editors. Pergamon Press, New York. 83–87.

Wikswo, J. P., Jr., P. C. Samson, and R. P. Giffard. 1983. A low noise, low input impedance amplifier for magnetic measurements of nerve action currents. *IEEE Transactions on Biomedical Engineering.* BME-30:215–221.

Woosley, J. K., B. J. Roth, and J. P. Wikswo, Jr. 1985. The magnetic field of a single axon: a volume conductor model. *Mathematical Bioscience.* 76:1–36.