brief communication

Magnetic field of a single muscle fiber
First measurements and a core conductor model

J. M. van Egeraat, R. N. Friedman, and J. P. Wikswo, Jr.

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

ABSTRACT We present the first measurements of the magnetic field from a single muscle fiber of the frog gastrocnemius, obtained by using a toroidal pickup coil coupled to a room-temperature, low-noise amplifier. The axial currents associated with the magnetic fields of single fibers were biphasic and had peak-to-peak amplitudes ranging between 50 and 100 nA, depending primarily on the fiber radius. With an intracellular microelectrode, we measured the action potential of the same fiber, which allowed us to determine that the intracellular conductivity of the muscle fiber in the core conductor approximation was 0.20 ± 0.09 S/m. Similarly, we found that the effective membrane capacitance was 0.030 ± 0.011 F/m². These results were not significantly affected by the anisotropic conductivity of the muscle bundle. We demonstrate how our magnetic technique can be used to determine the transmembrane action potential without penetrating the membrane with a microelectrode, thereby offering a reliable, stable, and atraumatic method for studying contracting muscle fibers.

INTRODUCTION

For a deeper understanding of the electric and magnetic fields produced by biological tissue, it is important to characterize the behavior of a single cell. Almost a decade ago, we reported the first measurements of the magnetic field of a nerve impulse (1). In this paper we present the first recordings of the magnetic field produced by action currents propagating in a single skeletal muscle fiber.

Electrical activity of biological tissue can be detected by a limited number of methods: electrical recording of action potentials, optical methods (e.g., birefrigence, voltage sensitive dyes), or magnetic recording of action currents. The first method has the advantage of a relatively high signal-to-noise ratio for typical biological signals, but requires the penetration of the cell with a microelectrode. The optical methods, while avoiding the adverse effects of penetration, have other limitations such as an uncertain origin of the signal (2) and long-term instability (photobleaching, dye washout [3]). In magnetic recording, the signal-to-noise ratio is usually not as good as that which is attainable with electrical measurements using intracellular microelectrodes; but, this disadvantage can be overcome by signal averaging. The magnetic technique is proving to be especially useful in situations where electric recordings are difficult or impossible, such as measurements of the axial variation of the membrane potential near the site of nerve crush injury, requiring multiple electrode penetrations (manuscript in preparation), in vivo recordings of action signals in human nerves (4), or studies of single muscle fibers whose contractions preclude stable microelectrode impalement.

Combined electric and magnetic measurements can be used to obtain information about the intracellular conductivity (σ) and the membrane capacitance (c_m) of the fiber. Alternatively, the magnetic recordings can be combined with known values of σ and c_m to determine the membrane potential without microelectrodes (5, 6).

Recordings from single muscle fibers form a necessary element in the analysis of compound action signals from bundles of simultaneously firing fibers. Wijesinghe (7) presents a model to decompose a magnetically recorded compound action current into the contributions of the individual fibers. Important parameters in such models are the intracellular conductivity and the shapes and magnitudes of the single fiber action currents. Once these data are known, it may be possible to use totally noninvasive, in vivo measurements with high-resolution SQUID magnetometers (8) to distinguish between motor units and to obtain data about the fiber composition of each motor unit, without the need for invasive, electrical measurements.

MATERIALS AND METHODS

In our experiments, we used the gastrocnemius muscle of the bullfrog (*Rana catesbiana*). The muscle was dissected free and submerged in frog Ringer's solution at 21°C. The connective tissue layers around the muscle were opened and a small bundle of 20–40 muscle fibers was carefully dissected from the muscle. The bundle was at least 20 mm

in length. One side of the bundle remained attached to the muscle and the other side was threaded through two toroidal pickup cores (9) connected to a pair of low-noise, current-to-voltage amplifiers (10, 11). The bundle was stretched to approximate its original length and fixed in the bath. The ferrite cores for detecting the magnetic field (type OW 40502; Magnetics, Butler, PA) each had inner and outer radii of 1.08 and 1.95 mm, respectively, and a width of 1.25 mm. The core was wound with 66 turns of insulated copper wire (40 gauge) and a single turn calibration winding. A thin layer of epoxy insulated the core from the bath. We used a microcomputer-based data acquisition system to average 512 recordings of the magnetic signal, thereby improving the signal-to-noise ratio by more than a factor of 20.

Electrical recordings were made with a micropipette, filled with 3 M KCl, coupled to an electrometer (WPI S-7071A; World Precision Instruments Inc., New Haven, CT). Typically, this technique would allow no more than 10 consecutive recordings of an action potential, because the contraction of the fiber would either eject the micropipette or damage the membrane seal around the pipette tip. Therefore, the electrical recordings were made only after recording the magnetic data. The magnetic recording is much less labile to motion artifacts, as there is no physical contact between the probe and the contracting fiber. Also, the magnetic recording will not be distorted by small axial displacements of the fiber relative to the probe, because the spatial extent of the action potential is at least one order of magnitude greater than the contractile movement of the fiber.

Although the toroid was threaded by a bundle, we measured the signal of only one fiber by selective stimulation. A single fiber was partly separated from the bundle so that it could be stimulated extracellularly, without firing any of the other fibers. Threshold was reached with a 60-μs, 0.7-mA stimulus pulse. A precise determination of the conduction velocity of the action potential was made by measuring the propagation delay between the two toroids of known separation.

We used a core conductor model to analyze our data (5). Ignoring volume conductor effects is valid as long as the effective radius (r_{eff}) of the toroid is smaller than the spatial length of the rising edge of the action potential (12). In our experiments, the length of the depolarization phase of the action potential was always >2.5 mm, whereas r_{eff} was 1.47 mm, as determined by the toroid dimensions (9).

In the core conductor approximation, the relationship between the axial current I_a, measured with the toroid, and the intracellularly-recorded membrane potential V_m, can be expressed as

$$I_a(t) = (1/R)(dV_m/dx) = (uR)^{-1}(dV_m/dt), \qquad (1)$$

where R is the resistance per unit length (Ω/m) of the fiber, u is the conduction velocity (m/s) and x is the axial coordinate. If u is known, R can be determined from the measured action potential and current. If we know R and u beforehand, it is possible to calculate V_m from I_a.

Eq. 1 assumes that we measure I_a at a single location along the fiber. In reality, however, the toroid is measuring the axial current, averaged over the width of the toroid. As a consequence, the measured signal is slightly broadened and has a lower amplitude than the actual I_a in the muscle fiber. The toroid acts as a moving average filter with a corresponding low-pass filter characteristic. The z-transform $H(z)$ of this filter as a function of the complex variable z is given by (13)

$$H(z) = (1/N) \sum_{n=0}^{N-1} z^{-n} = (1/N)(1 - z^{-N})/(1 - z^{-1}). \qquad (2)$$

N is an integer that determines the length of the summation, and is related to the width (d) of the toroid, the conduction velocity, u, and the sampling interval (t_s) by

$$N = d/(ut_s). \qquad (3)$$

In our case, t_s was 0.15 ms and u was 2.0 m/s, which leads to $N = 4$. Given this, we can correct for the systematic error introduced by the toroid by inverse filtering with a deconvolution filter $H^{-1}(z)$

$$H^{-1}(z) = N(1 - z^{-1})/(1 - z^{-N}). \qquad (4)$$

$N = 1$ leads to no correction because $H^{-1}(z)$ is equal to unity in that case.

The filter $H^{-1}(z)$ can reconstruct any signal that is distorted by the moving average filtering of the toroid, provided that the signal is free of noise. If this is not the case, the filter may resonate with a fundamental frequency $1/(Nt_s)$. We suppressed this resonance in two ways: first, we smoothed the measured signal by replacing it by a fourth order B-spline with 12 control points (14). The B-spline was fitted to the measured signal with a least squares algorithm and then deconvoluted with the filter given by Eq. 4. As expected, the resulting signal contained some resonance artifacts, evoked by the steep transition between the first and second phase of the magnetic signal. Therefore, the second step of resonance suppression consisted of a simple subtraction of a phase-shifted segment of the tail of the action current waveform (resonating around $I_a = 0$) from the second phase of the action potential that contained the same resonance.

Finally, the deconvoluted spline function, which represents I_a, was integrated to obtain the membrane potential. In this way, it was possible to reconstruct the action potential from the measured action current. Even if the second step of resonance suppression is omitted, the same

result is obtained, because of the smoothing effect of the integration.

RESULTS

Fig. 1 shows the recorded single fiber action current (a) and action potential (b) as solid lines. The action potential does not immediately return to the initial resting potential. This may be due to a long depolarizing afterpotential in the frog muscle (15), changes in the electrode impalement, or other electrode artifacts following the contraction. The prolonged afterpotential was not consistently observed in all fibers. The control points of the B-spline that was fitted to the action current are given in Table 1. A plot of the spline function was barely distinguishable from the measured signal and was omitted from Fig. 1 a. The dotted line in Fig. 1 a is the deconvoluted action current. The measured current is slightly wider and lower in amplitude than the deconvoluted signal because of the spatial averaging effect of the toroid.

The conduction velocity, u, was measured to be 2.0 ± 0.1 m/s. The measured action potential had a maximum slope of 92 V/s, occurring 0.55 ms before the peak of the action potential. At this time, the deconvoluted action current also reached its maximum of 71 nA. We estimate
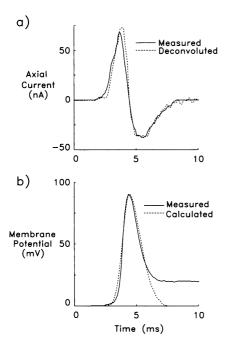

FIGURE 1. Action current and potential in a single muscle fiber of the frog gastrocnemius. (a) Recorded (solid) and deconvoluted (dotted) action current; (b) recorded (solid) and calculated (dotted) action potential. Low-pass filter at 15 kHz, 512 averages for the action current, no averages for the action potential.

TABLE 1 B-Spline control points for the fitted action current.

Control point no.	Time	Action current
	ms	nA
1	1.000	0.6
2	2.250	−6.7
3	3.000	23.9
4	3.375	46.9
5	3.750	77.7
6	4.125	43.1
7	4.500	−3.0
8	4.875	−32.8
9	5.250	−36.8
10	6.000	−37.6
11	7.500	4.3
12	10.000	−0.1

For a reconstruction of the B-spline, see reference 12.

the error in both the current and the time derivative of the voltage to be 10% or less. With these data, the resistance per unit length, R, was found to be 0.65 ± 0.18 GΩ/m. An extensive treatment of all sources of error is given in reference 12.

Based on these numbers, we could perform the integration to calculate the single muscle fiber action potential, shown as the dotted line in Fig. 1 b. Except for the tail of the intracellularly recorded action potential, the measured and reconstructed data match very well. The prolonged afterpotential may be physiological but undetected by the toroid as a result of the high-pass filter characteristics of a current transformer. Alternatively, the afterpotential may be an electrode artifact, as mentioned earlier.

The membrane capacity, C_m, per unit fiber length can be calculated from V_m and I_a, with the assumption that the radial membrane conduction current is negligible during the subthreshold phase of the action potential (5). We make the rough approximation that the capacity of the T-tubular system is included in the membrane capacity, or also that the so called access resistance of the T-tubular system is equal to zero (16–19). Valdiosera (19) found time constants for the T-tubular system in the range of 0.5–1.0 ms, which means that the approximation is acceptable for the relatively slow subthreshold phase of the action potential. The capacitive membrane current (J_c) per unit fiber length is given by

$$J_c = C_m(dV_m/dt). \qquad (4)$$

Continuity of current requires that

$$J_c = (1/u)(dI_a/dt). \qquad (5)$$

These two equations can be solved for C_m. At 1.4 ms before the peak of the action potential, the rates of change

of V_m and I_a are 3.4 ± 0.3 V/s and 64 ± 6 μA/s, respectively. Using the value for u as above, we found that membrane capacity per unit fiber length, C_m, was 9.4 ± 2.4 μF/m.

The fiber radius, a, was found by optical microscopy to be 50 ± 10 μm. Now we can convert C_m to the specific membrane capacitance c_m (F/m²) by dividing by $2\pi a$. Also, we can convert R to the specific axial resistivity r (Ωm) by multiplying by πa^2. For c_m, we find 0.030 ± 0.011 F/m² and for r, 5.1 ± 2.3 Ω·m. The corresponding axial conductivity σ is the reciprocal of r and is 0.20 ± 0.09 S/m.

Finally, we checked the validity of the core conductor approximation. The results can be affected by the finite dimensions of the toroid and by the fact that the active fiber is surrounded by an anisotropic bundle of inactive fibers instead of by a grounded, zero-resistance bath. We corrected for the finite toroid size by deconvoluting the action current. To take into account the effects of the anisotropic conductivity, we used the volume conductor model presented by Roth and Wikswo (6). This model calculates the magnetic field produced by a given axial current in the presence of an anisotropic medium around the fiber. Using a four-electrode method (20), we determined that the transverse conductivity of the bundle was approximately one half the longitudinal conductivity. From the volume conductor model, it followed that the anisotropy reduced the magnetic field at r_{eff} by <15%. This error is less than the uncertainty in the other parameters and is ignored in this analysis, although it would be possible to correct for this systematic error.

DISCUSSION

Fig. 1 a shows that it is possible to use a toroidal coil and a low-noise amplifier to record 50 nA action currents from a single muscle fiber. After correcting for the toroid effects, it is possible to accurately reconstruct the action potential from the measured action current. It is conceivable that a thinner toroid of higher permeability would eliminate the need for the deconvolution of the action current (9).

The intracellular conductivity of 0.20 ± 0.09 S/m, calculated from our magnetic and electric measurements, is significantly lower than that of nerve fibers. Proposing a structure or mechanism accounting for this difference would be speculative at this time; however, our results do not differ markedly from the findings of other investigators (15, 19, 21). The largest source of uncertainty is the fiber radius. Our value for the fiber resistance per unit length (0.65 ± 0.18 GΩ/m) is within the range of error of the value 0.46 ± 0.07 GΩ/m, found by Valdiosera et al. (19). Valdiosera did not measure the fiber radius inde-

pendently, so that a comparison of the intracellular conductivity cannot be made. Adrian and Peachey (15) and Hodgkin and Nakajima (21) used higher values (0.25–0.60 S/m) for muscle fiber intracellular conductivity than the value found in our studies. Based on our earlier analysis of a single nerve axon (12), the combined electric/magnetic measurement of the conductivity may have less than half the error of values derived from cable-constant measurements made with multiple microelectrode penetrations. Our value for the membrane capacitance is in agreement with values from experiments reported by others (18, 19). The fiber radius is again the largest source of error.

Recordings of the magnetic signature of a single muscle fiber, such as presented here, will form the basis for future analyses of magnetically recorded signals from fiber bundles. A decomposition of compound action currents into the contributions of the individual fibers is only possible if we know some of the basic characteristics of the single fiber.

With new, high-resolution SQUID magnetometers (4, 22), it is now feasible to analyze the magnetic signals from single motor units in vivo without the need for invasive measurements with needle electrodes. This may provide the clinician with data on the number and size of fibers in a motor unit or muscle. Furthermore, the values for the intracellular conductivity and membrane capacitance are important parameters in the study of muscle plasticity and in numerical models that simulate the propagation of action potentials. Such studies and models should improve our ability to interpret clinically measured magnetic signals and advance their use in diagnosis. After correcting for toroid effects, it is possible to accurately reconstruct the action potential from the measured action current, eliminating the need for penetration of the membrane and thereby offering a reliable, stable, and atraumatic method for studying contracting muscle fibers.

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