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Electrical impedance myography: Transitioning from human to animal studies

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Abstract

Objective: To determine the feasibility of performing electrical impedance myography (EIM) in rats.

Methods: EIM was performed on the hamstring muscles of 6 healthy adult rats with applied frequencies of 2–300 kHz. Studies were performed over a 6-week period, with 3 rats having recordings made from the skin (surface EIM) and 3 with recordings directly from the muscle (direct-muscle EIM). In addition, sciatic nerve crush was performed on one rat and comparisons made pre- and post-injury. Reactance and resistance were measured and the primary outcome variable, the phase angle (θ), calculated.

Results: EIM patterns in the rat hamstring muscles were qualitatively similar to those observed in human subjects. This held true for both surface and direct-muscle recordings, although direct-muscle data appeared less repeatable. Sciatic nerve crush data in the single rat showed a dramatic reduction in phase and a relative loss of frequency-dependence.

Conclusions: EIM data similar to that obtained from human subjects can be acquired from rat muscles with surface recordings proving more consistent and easier to obtain than direct-muscle recordings. Changes seen with sciatic nerve crush mirror those seen in patients with neurogenic injury.

Significance: These results support the possibility of performing EIM on rat models of neuromuscular disease.

Keywords: Electrical impedance; Rats; Neuromuscular disease

Electrical impedance myography (EIM) is a new non-invasive technique for the evaluation of muscle with potential use for the assessment of neuromuscular disease. In EIM, high-frequency, low-intensity electrical current is applied via surface electrodes to a limb and the resulting voltage pattern over a muscle or muscle group is evaluated (Shiffman et al., 1999; Rutkove et al., 2002). The parameters obtained include the resistance (R), which measures impedance associated with the passage of current through intra- and extra-cellular fluids, and reactance (X), which reflects impedance associated with passage of currents through the cell membranes. Both R and X depend on the shape and cross-sectional area of the tissue. However, these dependencies tend to cancel in the calculation of the phase, θ, obtained using the equation $\theta = \arctan X/R$ (Shiffman et al., 1999, 2001).

One advantage over conventional techniques, such as needle electromyography, is that the applied current cannot be sensed by the patient, since it is of low intensity and high frequency. While EIM published work to date has focused on single-frequency measurements, ongoing efforts have included measurements using multiple frequencies; this 'broadband approach' provides a richer impedance picture of the muscle than that obtained at a single frequency. This is demonstrated in Fig. 1 in which multifrequency-EIM in a typical healthy normal subject is compared to that obtained from a patient with amyotrophic lateral sclerosis. In this example, frequencies between 10 and 300 kHz were applied and the major outcome variable, the phase (θ), measured

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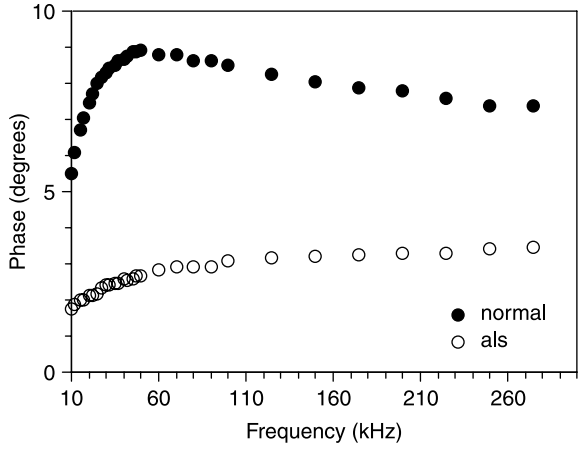

Fig. 1. Human multifrequency-EIM data, presented as phase (θ) vs. applied frequency, from the tibialis anteriors of two 69-year-old women, one healthy and one with ALS (S.B. Rutkove, personal communication).

across the tibialis anterior in both individuals. In addition to a reduction in the absolute phase at 50 kHz (the frequency of measurement typically used for single frequency studies), a substantial transformation of the entire shape of the curve also occurs, potentially providing a richer picture of the changes brought about by disease.

Earlier work in our group was initiated on human subjects since EIM is safe, but also because the larger muscles allow ready refinement of the technique and its implementation. However, despite making substantial progress in methodology and in demonstrating its potential use in disease assessment, certain aspects of EIM are not addressed effectively using this strategy. In particular, underlying EIM theory is based primarily on electric circuit analogs, in which the electrical parameters can be adjusted to model the impedances observed for diseased or healthy states. From a theory-development perspective, the human data have been challenging to work with given the heterogeneity of disease processes and, for obvious ethical and practical reasons, the limitations in obtaining pathological material from patients to correlate with the EIM findings. Moreover, there have been ongoing questions regarding the effect of the skin-subcutaneous fat layer on our measurements. For all of these reasons, we decided to evaluate the possibility of applying EIM to the hamstring muscles of the rat, both recording from the skin (surface EIM) and directly from the muscle (direct-muscle EIM). Once the technique is optimized in healthy rats, studies of diseased models can be systematically pursued. These would include models of neurogenic change (via sciatic nerve crush), myopathic disease, via experimental immune myositis (Matsubara and Takamori, 1987), and disuse, via hind limb suspension (Riley et al., 1990). Quantitative histomorphometry can then be performed on the muscle itself, with the goal of developing an improved understanding of how pathological changes relate to the observed EIM patterns.

In this study, we review the results from our first experiments in transitioning EIM from human beings to rats, with our aims being specifically: 1, to evaluate normal rat data to determine how it compares to human data; 2, to compare data obtained from surface vs. direct-muscle EIM; 3, to assess the potential repeatability of the technique and; 4, to preliminarily evaluate the effects of sciatic nerve crush on the EIM data.

1. Methods

A total of 6 male SASCO Sprague Dawley rats, approximately 50 days old with a weight of 181–200 g, were obtained from Charles River Laboratories, Wilmington, MA. They were housed in individual cages with filter tops in a temperature (72 °F) and humidity (40–70%) regulated environment on a 14 h light/10 h dark cycle, with food and water ad libitum. All work was approved by the Beth Israel Deaconess Medical Center review board for animal studies. One additional male rat also underwent sciatic nerve crush as will be described below.

The animals were randomly separated into the two groups, 3 to undergo surface EIM recordings and 3 to undergo direct-muscle EIM recordings. Each animal was studied on a weekly basis for a total of 6 weeks in order to evaluate the technique's potential repeatability. Prior to testing, each animal was anesthetized with 10 mg/kg xylazine and 80 mg/kg ketamine, administered intraperitoneally. Dosage was proportionately adjusted throughout the course of the study as the animals grew. An additional half-dose of ketamine was administered if the animal's level of anesthesia appeared to decrease during testing, with the depth of anesthesia being ascertained by intermittent tail pinches and evaluation of tail movement. In addition, blood flow, as determined by pinkness in the tail and feet, and respiration, were visually monitored. During EIM testing, the animals were kept under two heat lamps in order to sustain constant body temperature and ophthalmic lubricant was applied to the eyes to assuage dryness.

The technique of multifrequency-EIM is fundamentally the same as what has been used for single-frequency linear-EIM (Shiffman et al., 1999; Rutkove et al., 2002) except that current is injected at selected frequencies between 2 and 300 kHz. This introduces technical problems whose discussion would cloud the issues presented here, and which will be discussed separately (Shiffman et. al., in preparation). But briefly, impedances are measured using a computer controlled wide band lock-in amplifier (Signal Recovery Model 7280, Advanced Measurement Technology Inc., Oak Ridge, TN, U.S.A) coupled with an active, very low capacitance input probe (Tektronix model P6243, Beaverton, OR, U.S.A). Values of R and X are obtained for the chosen frequencies, f, and the computer plots and stores R(f), X(f) and from them the phase, q(f), for further analysis. Absolute accuracy of the phase values is not an important

issue for the present work, but evidence for the required consistency of the technique is implicit in the curves of Figure 4A below.

1.1. Surface EIM recordings

Once anesthetized, the animal was placed on its right side and the dorsal aspect of the proximal hind limb shaved. Depilatory cream was subsequently applied to ensure complete hair removal in that one area. Then, for testing, each animal was placed in a prone position with the limbs splayed out straight and perpendicular to the body. After alcohol was used to cleanse the entire area, 4 disposable sterile and self-adhesive Ag/AgCl electrodes cut to a length of 2 cm and width of 0.5 cm (Nicolet Biomedical/VIASYS Healthcare, #019-400400 or Neuroline Solid Gel Surface Electrodes#700 10-K/C, Ambu, Inc.) were placed along the left biceps femoris muscle of dorsal hind limb, perpendicular to the general direction of the underlying muscle fibers (Fig. 2). During the first week of testing, 8 black dots, evenly spaced 0.5 cm apart, were permanently tattooed on the skin along the muscle with the first mark placed at 0.5 cm from the spine and the last placed at the very distal part of the muscle. The 4 electrodes were placed at markers 1, 3, 5, and 7. The leads from the electrodes were attached directly to the low-capacitance probe of the lock-in amplifier. The electrode at tattoo marker 7 served as C1, the distal current electrode, and the electrode at marker 1 was C2, the proximal current electrode. The electrodes placed at markers 5 and 3 served as V1 and V2, the distal and proximal voltage electrodes, respectively. New, sterile electrodes were used for each animal tested.

1.2. Direct-muscle EIM recordings

Once anesthetized, hair on the hind limb was removed in the same fashion as in the surface EIM group. After an alcohol wipe was used to cleanse the area, the skin was cut in such a way that a flap of skin was lifted to expose the entirety of the biceps femoris. The 4 electrodes (Nicolet Biomedical/VIASYS Healthcare, #019-400400) were placed on the muscle as in the surface EIM group, except no tattooed markers were utilized (Fig. 3). Multifrequency-EIM recordings were performed in the same way as in the surface EIM group. After completion of testing at each study session, the skin was closed with silk sutures and antibacterial ointment applied to the wound. Sutures were removed 3 days after testing. Analgesic (0.1 ml of 0.1–0.5 mg/kg Buprenorphine) was administered intraperitoneally every 12 h for 2 days after testing. The same incision was opened and closed every subsequent week and electrodes placed in approximately the same location as earlier by careful re-measurement.

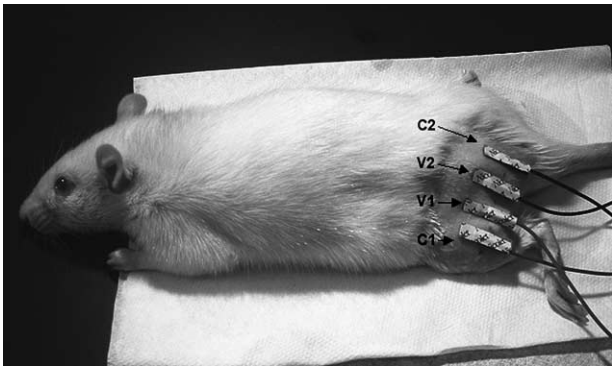

Fig. 2. Experimental set-up for surface recordings. C1 and C2 are the current-injecting electrodes and V1 and V2 are the voltage electrodes.

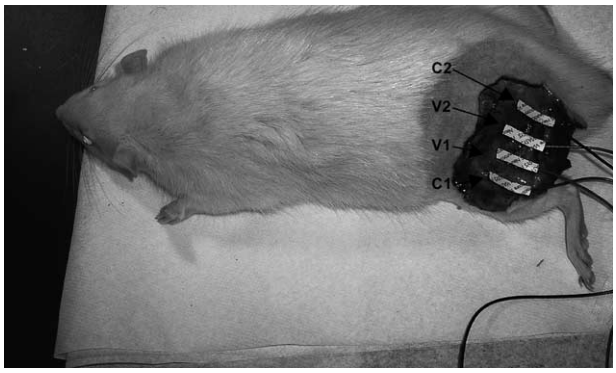

Fig. 3. Experimental set-up for direct muscle recordings. The same electrode naming system is used as in Fig. 2.

1.3. Sciatic nerve crush

One additional animal underwent identical surface EIM recordings immediately prior to sciatic nerve crush using a standard forceps for 30 s. Repeated surface EIM recordings were then made 15 days after injury. Given the substantial loss of muscle bulk that developed, the electrode array needed to be repositioned slightly in order to most effectively capture data from the atrophied area of tissue in the posterior compartment of the limb.

2. Results

Five of the six initial rats survived all 6 weeks of testing; one of the rats in the direct-muscle group died early in week 3 for unclear reasons, but presumably related to the weekly surgeries needed to expose the muscle. The data from that rat is excluded from this analysis. For all these analyses, we evaluate only the range of 10–300 kHz, as the data obtained below 10 kHz was erratic, likely reflecting capactitative effects of the electrode–skin/muscle interface.

2.1. Surface and direct-muscle recordings

Fig. 4a shows typical data for the 3 rats in the surface group, revealing that it is qualitatively similar to the normal

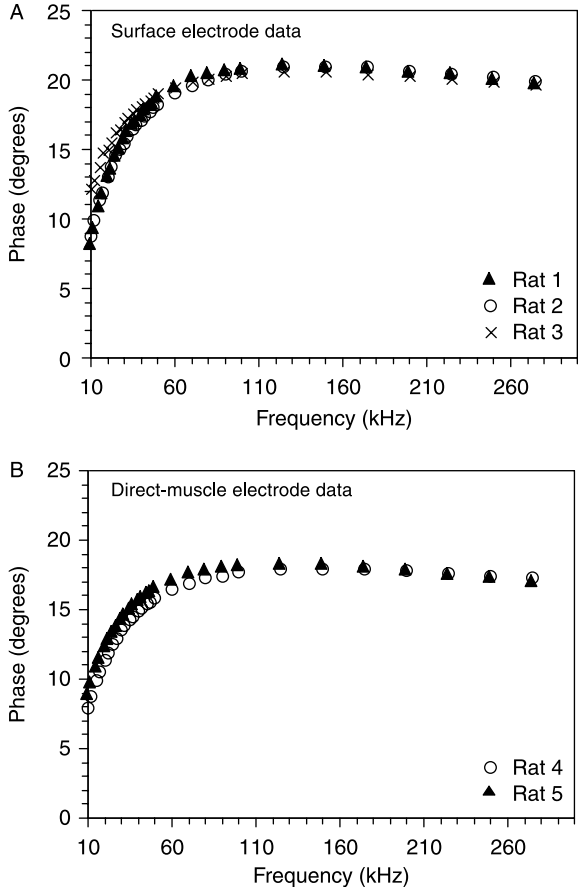

Fig. 4. Comparisons between typical data using surface and direct-muscle recordings. (A) Phase (θ) versus applied frequency from the 3 rats undergoing surface measurements. (B) The same plots from the two surviving rats undergoing direct-muscle measurements. In these examples, the surface data are qualitatively similar but of different value; however, the direct-muscle values were quite variable in general (see Fig. 5b), and little significance should be placed on their absolute values.

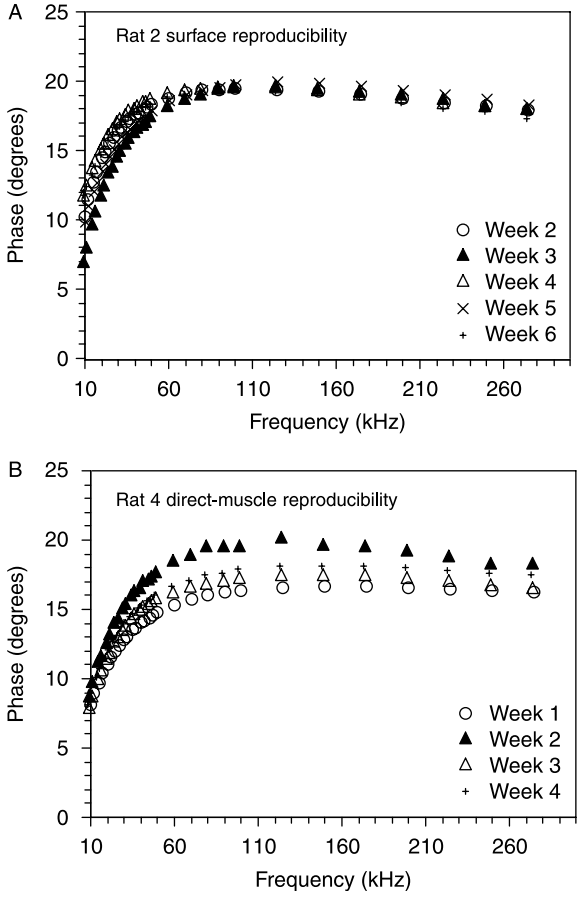

Fig. 5. Comparison of the examples of the best repeatability obtained between surface and direct-muscle techniques. (A) Measurements over a 5-week period in one rat using surface technique. (B) Measurements over a 5-week period in one rat using direct-muscle technique.

human data (Fig. 1 top), in that a gradual upslope at lower frequencies is followed by a peak and then a gradual descent as the frequencies increase. Unlike the human data, the peak is somewhat less prominent and occurs at a higher applied frequency (approximately around 120 kHz as compared to human's 50 kHz). The two surviving animals in the direct-muscle group also showed qualitatively similar data (Fig. 4b) to those of the surface group, although the curves were slightly lower.

2.2. Comparison of surface and direct-muscle recordings and potential repeatability

As Fig. 4 suggests, there does not appear to be any great added benefit in terms of single data collection comparing the surface and direct-muscle recorded data. The results are qualitatively similar and the substantial complexity, inconvenience, and unnecessary trauma to the animals would strongly argue against the direct-muscle approach. Nonetheless, if data from the direct-muscle group suggested better repeatability, there would be an impetus for pursuing such measurements. Fig. 5a and b shows the most consistent measurements we were able to obtain over a several-week time period for any one animal in each group. From this data, it appears that the repeatability of the surface method may be better, in fact, than with the direct-muscle method.

2.3. Sciatic nerve crush

The pre- and post-sciatic nerve crush data are shown in Fig. 6. The dramatic change in the both the shape of the curve and its relative value is similar to that observed in subjects with neurogenic disease, such as shown in Fig. 1 bottom.

3. Discussion

We have demonstrated that EIM can be applied to rats in an analogous fashion as to how it is used in human beings with qualitatively similar results. However, in much of our

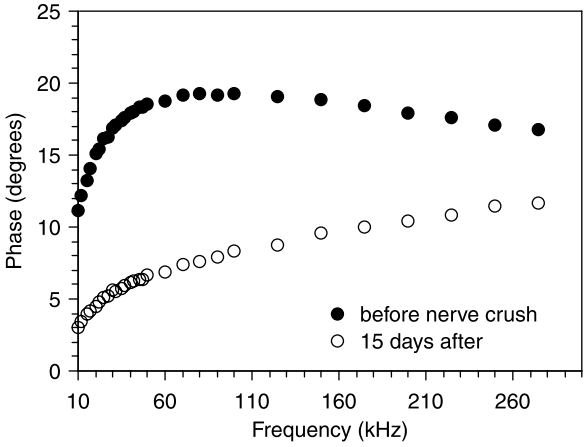

Fig. 6. Comparison of surface data obtained pre- and post-sciatic nerve crush. Note the overall lower phase at all frequencies and the substantial loss of the frequency-dependence.

human work to date, we have been using a somewhat different electrode set-up, in which electrical current is injected at a distance from the region of the voltage electrodes. For example, in the data collected in Fig. 1, the current electrodes were placed on both feet while the voltage electrodes were placed over quadriceps (what we have called the 'far current electrode montage'). However, in more recent work, we have been exploring the possibility of applying current in close proximity to the voltage electrodes ('the near current electrode montage') and are obtaining qualitatively similar results. The advantage of near-current electrode montage is that the current is 'forced' into a small region immediately underlying the voltage electrodes, thus providing information on the impedance characteristics of the muscle immediately beneath the voltage electrodes. With the far-current electrode montage, however, current flow is relatively parallel to the long axis of the limb and diffusely distributed throughout the tissues, thus providing data on the entire muscle mass beneath the voltage electrodes. Work on ALS and myopathy patients (Rutkove et al., 2002; Tarulli et al., 2005) supports the use of far electrode placement to assist with assessment of generalized disease. Near-current electrode placement may also be effective in this regard, but this arrangement also holds the promise of evaluating processes that affect muscles in a more restricted fashion, such as radiculopathy or mononeuropathy. Although we initially attempted to apply both far- and near-current electrode versions of EIM to the rat, the far electrode data, obtained by placing the current electrodes on the hind paws, was of poor quality; moreover, whether this truly represented a 'far-current electrode montage' was questionable, given that at most, the current electrodes were still only a few centimeters away from the voltage electrodes.

In this study, we chose to use modified versions of standard nerve conduction electrodes. These electrodes served the purpose well, although were somewhat awkward to use on smaller, younger rats, becoming easier to manipulate and place accurately as the rats grew. Much of the awkwardness involved the relative inflexibility of the wire lead coming from the electrode and the need to cut the electrode itself to the specified size. There are a number of other commercially available electrodes, including needle electrodes, that we will likely assess for consistency of measurements and general ease-of-use in the future, having confirmed the possibility for successful animal studies with this effort. A fixed frame to hold the electrodes and the wires in association with a retractor to assist in positioning the rat hind limb into the preferred position for study will also be helpful.

One purpose of this work was to determine the effect of the skin-fat layer on EIM measurements. Whereas our data here suggests little effect, the relevance of these results to human studies remains uncertain since the skin-fat layer in rats is only 2–3 mm as compared to as much as several centimeters in human subjects. Thus, the effect of skin-fat layer thickness on human EIM measurements will remain a focus of study. In fact, in our ongoing clinical work we routinely measure the thickness of the skin-fat layer using ultrasound and are in the process of assessing how variation in its thickness impacts the EIM data obtained.

In addition to assessing the feasibility of performing measurements in rats, we repeated the studies on a weekly basis to obtain some initial sense of the repeatability of the technique. As Fig. 5 implies, the data using surface electrodes appeared more consistent than the direct-muscle data. There are several reasons that likely explain this, including the fact that the repeat surgeries complicate the direct-muscle measurements. These surgeries introduce other variables that may substantially impact on the recorded data, such as local edema/inflammation, dehydration of the exposed muscle during measurement, and tissue cooling. Perhaps most importantly, placement of the electrodes on the skin via tattoo can be accomplished more precisely than by performing repeated distance measurements from a fixed point as is necessary with the direct-muscle studies.

Another issue impacting repeatability with all rat studies is that of growth, since over the 6-week time period of this study, the animals were continually increasing in size. For the surface studies, the tattoos served as markers, but clearly the amount of tissue underneath and between the electrodes increased throughout. Although this likely accounts for some of the changes observed in the EIM data, the variability did not occur in a consistent fashion (e.g the curves did not show a gradually increasing phase over time—see Fig. 5), making the inconsistencies more likely technological in origin rather than due to a growth effect. We anticipate substantial modifications to our methods as we gather more experience, and only when those refined methods are in place would a true reproducibility study be warranted. Finally, we note that while the absolute placement of the curve did shift, the relative shape of

the curve did not. This raises the possibility of using descriptors of the curve shape, rather than absolute values of maximal phase, for example, as a preferred method of data analysis.

Several other variations to the EIM technique could also be applied to rats, but were not addressed in this initial animal work. Specifically, the angle of the voltage and near current electrodes relative to the muscle fiber direction can be varied, such that the voltage and current electrodes can be placed in a line parallel with or perpendicular to the muscle fibers. Such a scheme has the ability to evaluate the anisotropy of the tissue, or the tendency for applied current to travel preferentially along the muscle fibers rather than across them (Aaron et al., 1997). Another variation would include performing EIM measurements during electrically-induced muscle contraction. An earlier study in humans (Shiffman et al., 2003) has shown that muscle contraction produces changes in the impedance signature, potentially providing additional information on muscle status.

As an added benefit to this work, with our improved ability to work with the small muscles of animals, it is possible that EIM measurements could be performed on established rodent neuromuscular disease models potentially providing useful outcome measures in pre-clinical animal studies. But ultimately, by being able to induce consistent neurogenic, myopathic, and disuse changes in rat muscle, we hope to be able to understand better the mechanism underlying the impedance changes we have observed thus far in our investigations in human neuromuscular disease.

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