A flexible electrode array for muscle impedance measurements in the mouse hind limb: A tool to speed research in neuromuscular disease

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Abstract. Electrical impedance myography (EIM) is a bioelectrical impedance technique focused on the assessment of neuromuscular diseases using tetrapolar surface arrays. Recently, we have shown that reproducible and sensitive EIM measurements can be made on the gastrocnemius muscle of the mouse hind limb and that these are sensitive to disease alterations. A dedicated array would help speed data acquisition and provide additional sensitivity to disease-induced alterations. A flexible electrode array was developed with electrode sizes of 1mm X 1mm by Parlex, Inc. Tetrapolar electrode sets were arranged both parallel to (longitudinal) and orthogonally to (transverse) the major muscle fiber direction of the gastrocnemius muscle. Measurements were made with a dedicated EIM system. A total of 11 healthy animals and 7 animals with spinal muscular atrophy (a form of motor neuron disease) were evaluated after the fur was completely removed with a depilatory agent from the hind limb. Standard electrophysiologic testing (compound motor action potential amplitude and motor unit number estimation) was also performed. The flexible electrode array demonstrated high repeatability in both the longitudinal and transverse directions in the healthy and diseased animals (with intraclass correlation coefficients of 0.94 and 0.89, respectively, for phase angle measured transversely). In addition, differences between healthy and diseased animals were identifiable. For example, the 50 kHz transverse phase angle was higher in the healthy as compared to the SMA animals (16.8° ± 0.5 vs. 14.3° ± 0.7, respectively) at 21 weeks of age (p = 0.01). Differences in anisotropy were also identifiable. Correlations to several standard neurophysiologic parameters also appeared promising. This novel flexible tetrapolar electrode array can be used on the mouse hind limb and provides multidirectional data that can be used to assess muscle health. This technique has the potential of finding widespread use in the evaluation of drug therapies in neuromuscular animal disease models.

1. Introduction
Generalized neuromuscular diseases encompass a wide variety of disorders ranging from amyotrophic lateral sclerosis (ALS) to muscular dystrophy to generalized polyneuropathy. A theme common too many of these disorders is that there is no readily available therapy and that much basic research continues to rely on animal models. In fact, for many of these disorders one or more animal models have been developed. For example, in the case of ALS, while one genetic model has been used for years [1], other models become available as new genetic causes for the disease are identified [2, 3].

In addition to seeking mechanisms underlying the pathogenesis of these diseases, these models are also used for preclinical drug testing [4]. Such drug testing is used to determine preliminarily the safety of the drug, the doses needed, and the degree of therapeutic benefit offered. Current approaches for evaluating these diseases include functional measures, such as animal grip strength or walking speed as well as electrophysiologic measures [5, 6].
We have been exploring the development of new methods for assessing muscle function in these animals based on localized measurements of muscle impedance via electrical impedance myography (EIM) [7]. As part of that effort we initially began by studying larger rodents (rats) using a modified approach of that which we had been performing on humans [8]. However, for several reasons, the rat model is limited in value and thus we began performing measurements on mouse disease models, focusing the gastrocnemius-soleus muscles. Recent work showed that a fixed stainless steel 4-electrode array provided repeatable data and was also able to differentiate normal from ALS-affected muscle [9]. However, in an effort to improve our impedance measurements, we developed a multielectrode array capable of measuring in multiple directions relative to the major muscle fiber direction. Here we test that array in a group of healthy animals as well as a group with spinal muscular atrophy (SMA), a genetic pediatric neuromuscular disease that causes generalized weakness.

2. Methods

2.1. Mice
All studies were approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee. Breeding colonies of SMA (strain: B6.129-SmnlIms5(SmnI/SMN2)Mrph/J) mice were established from animals obtained from Jackson Laboratories (Bar Harbor, Maine). SMA mice were genotyped by tail snip and were weaned at 28 days. A total of 7 SMA mice and 11 littermate controls (control) were studied at 21 weeks of age.

2.2. Mouse setup
Animals were placed under 1% isoflurane anesthesia delivered through a nose cone with a heating pad underneath the limb to maintain consistent temperature. A depilatory agent (Nair face cream, Princeton, NJ) was applied to the skin to remove all fur; the skin was cleaned with 0.9% saline solution. The leg and animal were positioned as previously described [9].

2.3. Electrical impedance measurement
A tetrapolar electrode array was developed in conjunction with Parlex, Inc, (Methuen, MA). Figure 1a shows the electrode array in comparison to a penny. It is small enough to fit over the gastrocnemius muscle. In addition, as shown in Figure 1b, a schematic of that array, both longitudinal (current flowing along the muscle fiber direction) and transverse (the current flow perpendicular to the muscle fiber direction) configurations can be measured with the combinations of using different current and voltage electrodes. In this study, the set of current electrodes 1 and 2 and voltage electrodes 1 and 2 was utilized in the transverse direction; while the longitudinal direction was made by using current electrodes 3 and 4 and voltage electrodes 3 and 4. The circles are electrical vias connecting the contact side (top) with the electrode side (bottom). EIM measurements were performed with the EIM Vet1102 1kHz-10 MHz system designed by Convergence Medical Devices, Inc, (Medford, MA).

![Figure 1. Views of the electrode array. (a) The entire view of the electrode in comparison to a penny; (b) A close-up view of the tetrapolar electrode array. VE: voltage electrode; CE: current electrode.](image)

2.4. Standard electrophysiology
Compound motor action potential (CMAP) and motor unit number estimation (MUNE) were performed using a TECA Synergy T2 EMG Monitor System (Viasys, Madison, WI) on the left hind limb stimulating the sciatic nerve at the sciatic notch and recording via disposable ring electrodes around the entire distal leg, as previously described [10].
2.5. Reproducibility measurements
For assessment of technique repeatability, after an initial set of measurements was made, the electrode array was lifted up from the animal, and then replaced to the leg surface; the measurements repeated.

2.6. Data analysis
Reproducibility was assessed by calculating intra-class correlation coefficients (ICC). All data were summarized as mean ± standard error. Anisotropy difference is defined as the difference between transverse and longitudinal directions. Unpaired t-tests were used to compare the means between groups; Spearman correlation was used to assess the relationship between EIM phase and CMAP amplitude. For all analyses, significance was accepted at p < 0.05, two-tailed.

3. Results

3.1. Reproducibility
Excellent reproducibility was obtained with this technique in control and SMA mice at 50 kHz on both longitudinal and transverse configurations. Test-retest plots for one of the major EIM parameters, phase, are shown in Figure 2 a-d. High values of intra-class correlation coefficients (ICC) from the 2 groups of animals were achieved in both directions; for example, in transverse direction, the ICC values for control and SMA mice were 0.94 and 0.89 respectively.

3.2. EIM differences between control and SMA animals at 50 kHz
As shown in Figure 3 a-f, smaller values for EIM parameters and CMAP amplitude were found in the SMA animals as compared to the control animals. However, no difference for MUNE or girth was found between the 2 groups of animals. For example, transverse phase was 14.3° ± 0.7 for the SMA animals and 16.8° ± 0.5 for the control animals (p = 0.01). In addition, the anisotropy difference for phase was 5.2° ± 0.4 in the control animals and 3.9° ± 0.3 (p = 0.02) in the SMA animals. CMAP amplitude showed significantly differences between the 2 groups animals (44.5 ± 6.3 mV for the SMA mice and 62.4 ± 4.3 mV for the control mice, P = 0.04). However, MUNE for the control mice (61.3 ± 24.9 motor units) was not different from the SMA animals (26.0 ± 3.3 motor units) with p as 0.19. Neither was girth (6.9 ± 0.1 mm for control mice vs. 6.8 ± 0.1 mm for SMA mice, p = 0.39).

3.3. Correlation between transverse phase and CMAP amplitude
Spearman correlation coefficient was 0.68 with p as 0.09 for the 7 SMA animals.
4. Discussion

These results show that a flexible 4-electrode array can provide EIM data that is reproducible both in the longitudinal and transverse directions. These reproducibility values are similar to those observed for rigid electrodes, as previously described [9]. In addition, the data can differentiate normal animals from those with SMA. Finally, the data appear to correlate with the CMAP amplitude, a standard measure of nerve and muscle function. Taken together, these results suggest that such a flexible electrode can differentiate normal muscle from that affected by SMA.

The main goal of this work was not to demonstrate the value of the technique for drug evaluation, since in order for that to be true, we would need to include a therapeutic intervention, which was not performed in this instance. However, doing so would be a logical next step, comparing the EIM data obtained to standard function, electrophysiologic and possibly pathological data.

One major advantage over other forms of electrophysiologic testing is that EIM is likely to be sensitive to conditions where standard electrophysiologic measurements would be expected to be relatively insensitive such as in myopathy or disuse atrophy [11, 12].

In summary, these data support the potential value of a flexible electrode array in animal testing, providing high-quality, repeatable data that can be used by scientists investigating disease mechanisms and by pharmaceutical companies seeking data as to potential drug efficacy.

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5. References
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