Measuring the multi-frequency electrical impedance of the mouse gastrocnemius muscle using a tetrapolar technique

J Li, P M Fogerson and S B Rutkove
Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, TCC-810, Boston, MA, 02215, USA

E-mail: srutkove@bidmc.harvard.edu

Abstract. Electrical impedance methods can be used to evaluate and monitor neuromuscular disease states. Recently, we have applied tetrapolar surface electrical impedance methods to the gastrocnemius muscle of the rat for this purpose and substantial changes in the impedance parameters after sciatic nerve crush can be identified. In order to be able to study additional animal models of nerve and muscle disease, however, it would highly desirable to be able to perform such impedance measurements in the mouse. Yet the small size of the mouse presents a substantial technical challenge. In this study, we evaluate a basic approach for performing such measurements. A series of thin, stainless steel strip electrodes affixed to the gastrocnemius and interfaced via a separate connector to the Imp SFB7® (Impedimed, Inc), provided an effective means for obtaining impedance data in the 20-500 kHz range. After two weeks, test-retest reproducibility was good, with intra-class correlation coefficients as high 0.84 and variability as low as 12.86 ± 6.18% in the 15 mice studied. Using this approach, it may now be possible to study impedance changes in a variety of mouse models of neuromuscular disease, including amyotrophic lateral sclerosis, spinal muscular atrophy, muscular dystrophy and Charcot-Marie-Tooth disease.

1. Introduction
Electrical impedance myography (EIM) is an impedance-based technique for the non-invasive assessment of muscle [1]. Earlier EIM work on human [2] and rats [3] has revealed that EIM holds the promise of serving as an indicator of neuromuscular disease progression while also potentially providing diagnostic information. However, to further broaden the application of EIM to this area, it would be ideal to be able to extend impedance measurements to the mouse, as many additional mouse models of neuromuscular diseases are available, including muscular dystrophy, spinal muscular atrophy, various forms of amyotrophic lateral sclerosis, and hereditary polyneuropathies (e.g., Charcot-Marie-Tooth disease). Thus, in this study, we assess a basic technique for performing localized impedance measurements on the gastrocnemius muscle in a group of healthy, adult mice.

2. Methods

2.1. Mice
All animal work was approved by the Institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Center, Boston, MA. Fifteen healthy male Swiss Webster mice at 12-weeks of age

1 To whom any correspondence should be addressed.
were obtained from Charles River Laboratories, Wilmington, MA and acclimated for one week after arrival at our animal facility before any testing was initiated.

2.2. Mouse setup
For consistency, the left hind limb of all the mice was used for all studies. After being placed under isoflurane anesthesia delivered via vaporizer, the mouse was placed in a prone position (see Figure 1 of [4]) with its left leg outstretched at approximately a 45° angle away from its body. The mouse was kept in this position by a retractor attached to a piece of cloth with a hole through which the left hind limb was slipped. The foot of this leg was taped down. This overall approach is similar to that being performed in rats [4]. A depilatory cream was applied to the distal left hind leg for 90 s to remove fur. A heating lamp above the body and a heating pad under the body were applied to maintain the body temperature at 37° C (surface temperature being monitored continually). A pinpoint tattoo was placed close to the center of the gastrocnemius muscle at a point approximately 2/3 of the distance from the midpoint of the lumbar spine and 1/3 of the distance from the base of the heel pad of each mouse. The purpose of the tattoo was to assist in repeat placement of electrodes.

2.3. Tetrapolar electrode array
The tetrapolar electrode array consisted of 20 mm long and 2 mm wide stainless steel strips, with wires soldered onto the ends that extended a short distance to a set of connectors that were interfaced to the impedance-measuring device. The four strips were placed in a line onto the adhesive surface of medical adhesive tape (3 M Micropore, 3 M Health Care, St. Paul, Minnesota) with an inter-electrode separation distance of 2 mm. As is standard in tetrapolar measurements, the two middle electrodes served as voltage electrodes and the outer two electrodes served as the current-injecting electrodes. Distilled water was used to clean the stainless steel strips between EIM measurements.

2.4. Electrical impedance measurement
Gauze, damp with 0.9% saline, was lightly dabbed on the leg skin to prepare the area prior to measurement. After waiting 30 s for excess saline to evaporate, the four-electrode array was placed along the length of the leg, centered about the tattoo. In this way, the proximal current electrode was close to the knee-joint.

Data were collected using the Imp SFB7® bioimpedance spectroscopy device (ImpediMed, San Diego, CA), in a range from 4 kHz to 1 MHz, although for the analyses that follow, only values from 20 kHz to 500 kHz were utilized.

2.5. Weighting
In addition to testing with adhesive tape alone (0 g), external weights of 6 g, 12 g, and 24 g, each with a footprint of 39 mm x 18 mm, were also placed on top of the electrode array. In addition to potentially improving electrode contact, the weighting also flattened out the measurement surface to some extent thus reducing interfering geometric inconsistencies. The weights were thoroughly insulated with non-conductive tape.

2.6. Reproducibility measurements
Reproducibility was conducted and examined at three different time points. For immediate reproducibility, the electrode array was detached and the animal removed from the set-up entirely, picked up, and weighed briefly on a nearby scale. The animal was then immediately brought back to the testing area and set up again with cleaned electrodes and the measurements repeated. Reproducibility was also measured at 1 day and at 2 weeks. During each series of reproducibility experiments, the first measurement was always the one without weight, followed in order of increasing weight. Based on our immediate reproducibility data, however, we excluded the 6 and 24 g weights from the 1-day and 2-week reproducibility studies.
2.7. Data analysis

Several different impedance parameters were utilized. First, we assessed the 100 kHz resistance ($R_{100}$), 100 kHz reactance ($X_{100}$), and 100 kHz phase ($P_{100}$). In addition, we were interested in evaluating the potential benefit of using “collapsed” parameters that attempt to represent the character of the multifrequency spectrum in a single value. One was the log-resistance slope (i.e., LogRslope), calculated by taking the resistance data from 20-500 kHz, performing a log transformation of both the measured values and the applied current frequencies, plotting the points, and taking the slope of a linear regression fitting of those points. The other was the phase slope (i.e., Pslope), obtained by performing a linear regression of the phase values in the 20-100 kHz range and calculating its slope generally showing a near-linear rise in values over that range. (The corresponding reactance slope was more variable and is thus not reported here.)

Reproducibility was assessed by calculating intra-class correlation coefficients (ICCs) and mean variability in per cent (as calculated by taking the absolute value of the difference of the first and second values and dividing by their mean x 100).

3. Results

3.1. Immediate reproducibility

Tables 1 and 2 provide the ICCs and mean variability (± standard deviation for the 15 animals studied), respectively, for the immediate reproducibility measurements. As can be seen, weighting substantially improve the reproducibility of the measurements, with only minor differences between the different weights being observed. Except for the Pslope, all the parameters have comparable variability.

| Weight | $R_{100}$ | $X_{100}$ | $P_{100}$ | LogRslope | Pslope |
|--------|----------|----------|----------|-----------|--------|
| 0 g    | 0.66     | 0.37     | -0.15    | -0.23     | 0.51   |
| 6 g    | 0.81     | 0.78     | 0.77     | 0.80      | 0.79   |
| 12 g   | 0.77     | 0.79     | 0.80     | 0.83      | 0.85   |
| 24 g   | 0.73     | 0.79     | 0.83     | 0.81      | 0.82   |

| Weight | $R_{100}$ | $X_{100}$ | $P_{100}$ | LogRslope | Pslope |
|--------|----------|----------|----------|-----------|--------|
| 0 g    | 7.51 ± 9.67 | 18.14 ± 20.83 | 16.12 ± 23.39 | 16.12 ± 23.66 | 34.41 ± 31.49 |
| 6 g    | 4.67 ± 7.21 | 13.92 ± 14.01 | 10.37 ± 9.48 | 9.49 ± 8.80 | 24.39 ± 24.71 |
| 12 g   | 5.20 ± 6.57 | 12.41 ± 16.13 | 8.93 ± 11.02 | 8.12 ± 10.60 | 23.24 ± 26.07 |
| 24 g   | 5.26 ± 6.53 | 12.40 ± 18.06 | 8.96 ± 12.41 | 8.94 ± 13.59 | 25.20 ± 31.53 |

3.2. One-day reproducibility

Table 3 provides the 1-day reproducibility data for 15 mice, now using only the 0 and 12 g weights. As anticipated, the variability is greater than in the immediate reproducibility studies.

| Weight | $R_{100}$ | $X_{100}$ | $P_{100}$ | LogRslope | Pslope |
|--------|----------|----------|----------|-----------|--------|
| ICC (0 g) | 0.40     | 0.42     | 0.54     | 0.60      | 0.32   |
| Variation (0 g) | 17.97 ± 11.16 | 32.15 ± 24.36 | 20.19 ± 16.99 | 18.70 ± 16.69 | 82.13 ± 102.81 |
| ICC (12 g) | 0.45     | 0.65     | 0.73     | 0.77      | 0.79   |
| Variation (12 g) | 17.15 ± 9.46 | 28.73 ± 22.42 | 17.32 ± 15.16 | 16.49 ± 14.42 | 50.35 ± 74.33 |

3.3. Two-week reproducibility

Table 4 provides the 2-week reproducibility data for the 15 mice. Interestingly the values are somewhat better than those observed at 1-day, with slightly higher ICCs and lower variability.
Table 4. Summary of ICCs and mean variability for 2-week reproducibility.

|          | R₁₀₀ | X₁₀₀ | P₁₀₀ | LogRslope | Pslope |
|----------|------|------|------|------------|--------|
| ICC (0 g)| 0.56 | 0.48 | 0.54 | 0.49       | -0.11  |
| Variation (0 g) | 13.79 ± 10.26 | 23.19 ± 19.07 | 15.04 ± 11.86 | 16.17 ± 12.82 | 77.25 ± 96.40 |
| ICC (12 g)| 0.55 | 0.73 | 0.84 | 0.84       | 0.43   |
| Variation (12 g) | 13.07 ± 9.67 | 21.20 ± 13.38 | 12.86 ± 6.18 | 13.12 ± 6.14 | 64.21 ± 71.67 |

4. Discussion

These results demonstrate that by using a basic tetrapolar set-up of four electrode strips placed over the mouse gastrocnemius muscle, we are able to attain relatively good reproducibility of measurements for a period as long as two weeks. Although the reproducibility is not as good as that achieved in humans [5] or in rats [4], it is likely sufficient and may possibly be further improved by additional modifications. The collapsed LogRslope parameter faired well, although Pslope was less consistent. Other work (unpublished results) in humans, appears to suggest good consistency to these other collapsed parameters, so it is not clear why the results here were not as reproducible.

In most of our work to date, we have relied up on disposable, adhesive Ag-AgCl electrodes. However, such electrodes cannot be utilized in the relatively confined space of the mouse hind limb. One obvious advantage to using stainless steel strips is that they are reusable. A disadvantage is the edges are difficult to smooth and irritate the animal’s skin more readily. It is likely this skin irritation that caused the 1-day reproducibility to be somewhat worse than the 2-week reproducibility. At two-weeks, the animals’ skin had entirely recovered from the earlier measurements. A variety of other reusable materials may be less injurious to the skin and these are being studied.

The weighting also appeared to help. It is most likely that this improved the contact between the relatively inflexible stainless steel strips and the mouse skin. However, it is possible that the weighting also helped to smooth out variations in the shape in the posterior leg muscles due to slight inconsistencies in positioning of the animal.

In conclusion, it is possible to obtain good reproducibility of localized impedance measurements from the mouse gastrocnemius muscle over periods as long as two-weeks. The technique may be useful for the assessment of mouse models of neuromuscular disease and warrants further refinement and application.

5. References

[1] Rutkove S B 2009 *Muscle Nerve* 40 936
[2] Esper G J, Shiffman C A, Aaron R, Lee K S and Rutkove S B 2006 *Muscle Nerve* 34 595
[3] Ahad M A, Fogerson P M, Rosen G D, Narayanaswami P and Rutkove S B 2009 *Physiol. Meas.* 30 1415
[4] Ahad M A and Rutkove S B 2009 *Clin. Neurophysiol.* 120 1534
[5] Rutkove S B, Lee K S, Shiffman C A and Aaron R 2006 *Clin. Neurophysiol.* 117 1244

Acknowledgments

This work was funded by Grant R01055099 from the National Institutes of Health.