Published in final edited form as:

Am J Phys Med Rehabil. 2009 July ; 88(7): . doi:10.1097/PHM.0b013e31819c575b.

Methodology to Obtain a Mixed Sural Nerve Recording in Mice

Mayisha W. Dunham, MD, MBA¹, Sarah E. James, PhD², Teresa E. Lever, PhD³, Qun Lu, PhD⁴, and Monica J. Carrion-Jones, MD⁵

¹ Department of Physical Medicine and Rehabilitation, Brody School of Medicine at East Carolina University, Greenville, NC 27834

² Department of Anatomy & Cell Biology, Brody School of Medicine at East Carolina University, Greenville, NC 27834

³ Department of Communication Sciences and Disorders, East Carolina University, Greenville, NC 27834

⁴ Department of Anatomy & Cell Biology, Brody School of Medicine at East Carolina University, Greenville, NC 27834

⁵ Department of Physical Medicine and Rehabilitation, Brody School of Medicine at East Carolina University, Greenville, NC 27834

Abstract

There is increasing interest in using mouse models for electrodiagnostic research. Studying transgenic mice with various pathologies, add to our knowledge of the natural history of a disease. It is imperative, however, to compare disease models to the appropriate control. Therefore, in order for these animals to be used in electrodiagnostic research, reference values must be set. If reference values are not available, the validity of data is highly questionable. We propose a method of obtaining mixed nerve action potentials and collect reference values from the sural nerve of mice. The results were a mean peak latency of 1.74 ms on the right and 1.89 ms on the left. The mean amplitude was 17.0 μ V on the left and 21.6 μ V on the right. In future studies, these reference values can be useful tools in analysis of murine subjects.

Keywords

Sural Nerve Action Potential; Mice; Cutaneous Suræ Caudalis; Peak Latency; Peak to Peak Amplitude

INTRODUCTION

Biochemical, immunohistochemical, and genetic studies have been utilized as tools to obtain information from mouse models. Phenotypically, mice may not exhibit the same features as seen in humans. For example, the mdx transgenic mouse was initially labeled as a mouse model of human Duchenne muscular dystrophy. It was after electrodiagnostic testing that it was found to be a model of primary, nonmyotonic myopathy.¹⁻³ This points to the importance of using electrodiagnosis in neuromuscular research. Increasingly, electrodiagnostic evaluation is being performed in mice, which requires the establishment of reference values. Sciatic motor unit action potential reference values have been recorded⁴ as well as action potential duration of fetal mouse dorsal root ganglia *in vitro*.⁵

Correspondence: Mayisha W. Dunham, MD, MBA Department of Physical Medicine and Rehabilitation, Brody School of Medicine at East Carolina University, 600 Moye Blvd, Greenville, NC 27834, (252) 847-7907 office, (252) 847-0840 fax.

The sural nerve is present in both humans and mice and its anatomical location makes it ideal for electrodiagnostic research. To our knowledge, the reference values for the sural nerve in mice have not been established. In the present study, we have developed a method to obtain mixed nerve conduction reference values for the murine sural nerve.

MATERIAL AND METHODS

Animals

Eleven wild type C57BL6 mice (23-43 g; 2-7 months old, Charles River) were used in this study. All experiments were approved by the Animal Care and Use Committee of our institution. Mice were maintained in group housing under a twelve hour light and dark cycle and allowed chow and water ad libitum.

Sural Nerve Action Potential Recordings

Four mice were anesthetized using Ketamine-Xylazine (.09 ml/10 g body weight) intraperitoneally. Seven mice were anesthetized using 2-3% isoflurane vapor. Antibiotic cream was applied to the eyes to prevent drying during anesthesia. The dorsum of the hindlimb was shaved to give better contact with the electrodes. The mouse was placed prone on a heating pad controlled by a rectal thermistor, (Frederick Haer Company Inc., Bowdoin, ME) ^{6, 7} and the body temperature was maintained between 36.5° C to 38° C ^{8,9} during the entire recording. A ground electrode, an unshielded Ag-AgCl electrode with contact posts (Biopac Systems, Inc, Goleta, CA) was placed underneath the abdominal area of the mouse.

As shown in Figure 1, near nerve pickup with antidromic technique was performed. Stainless steel annealed wire of 0.008 inch diameter (A-M Systems Inc, Carlsborg, WA.) was used to establish the active and reference electrodes. The wire was formed into a loop around the most proximal aspect of the animal's hind paw and then attached to a micrograbber (Biopac Systems, Inc). This represented the active electrode. The wire around the skin and at the connection with the micrograbbers was covered with conduction gel (Parker Laboratories, Fairfield, NJ). The reference electrode was placed 5 mm distal to the active electrode. The anode and cathode were 1.2 cm stainless steel needles (Faith Medical, Steedman, MO) fixed 1 mm apart and inserted subcutaneously, lateral to the knee. This site was determined after dissection and gross appreciation of the cutaneous surae caudalis ¹⁰, which is the nerve targeted in this study. The cathode was 1.5 cm proximal to the active recording electrode.

Figure 1. Depiction of antidromic technique using near nerve stimulation.

Obtaining Mixed Action Potentials

Action potentials were recorded with a TECA Synergy electrodiagnostic machine (Oxford Instruments, Oxfordshire, UK). The low and high frequency filters were set at 20 Hz and 3,000 Hz, respectively. ¹¹ Stimulation was started at an intensity of 1 mA and increased until the mixed nerve action potential peak to peak response was maximized. Stimulation duration remained at 0.02 ms. A mixed recording was expected because the likelihood of the sural nerve containing motor fibers has been well established in other small animal studies. ¹²⁻¹⁷

The amplitude (peak to peak) and latency (first negative deflection) of ten tracings were then signal averaged to minimize background noise. ¹⁸ As shown in Figure 2, low level compound muscle action potentials (CMAP) appeared after the mixed action potential secondary to slower conduction and synaptic delay. ⁶

Figure 2. Signal averaged tracing from the cutaneous surae caudalis. 1 is onset latency, 2 is peak latency, and 1-3 is the duration of the mixed action potential.

The mice also underwent sensory threshold analysis using Semmes-Weinstein filaments (Stoelting, Wood Dale, IL). We used this test to demonstrate the animals in this investigation had normal hind limb sensory thresholds. Animals were placed in a clear plastic chamber at room temperature and allowed to adjust to the environment for 10-15 minutes. Filaments ranging from 0.008-300 grams were applied to each hind paw five times while the mouse is placed on a screen. The filaments were applied to the foot pad until bending, and the filament that elicited a withdrawal response was recorded. The threshold response was determined by the filament that produced a withdrawal response greater than 50% (3 out of 5 trials). The threshold values were averaged for each limb.

Raw data was subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS; version 15.0).

RESULTS

The mean peak latency was 1.74 ms right hind limb and 1.89 ms for left hind limb with standard errors (SE) of 0.13 and 0.09, respectively. Mean peak to peak amplitudes were 21.58 μ V right and 17.00 μ V left with SE of 2.16 and 1.33, respectively. Mean sensory threshold values were 0.1 grams with a SE of 0.01 for both hind limbs.

Paired samples t-tests showed that there was no significant difference in the mean amplitude ($p=0.068$) and latency ($p=.105$) values obtained from left versus right hind limbs.

Figure 3. Mean amplitude and mean latency measurements using side to side comparison. The measurements in the side to side comparisons were not significantly different.

Independent samples t-tests revealed that the onset, latency, and peak to peak values were not significantly different ($p>0.05$) between the two types of anesthesia (isofluorane, $n=7$; ketaminexylazine, $n=4$) used in this investigation.

DISCUSSION

Evaluation of our results indicates that left and right hind limb amplitude and latency values showed no significant differences. Our technique and recordings correlate well with those of George and Bostock which were obtained using a rat model. As seen in George and Bostock's study, we were also able to separate out the CMAP waveforms. This lends to the reliability and reproducibility of our results. Also, our data suggests the recordings were not affected by anesthetic agent choice. Thusly, allowing flexibility of anesthesia choice for future studies using this electrodiagnostic method.

A potential problem in our methodology was the small sample size. This was addressed with repeated electrodiagnostic testing on the same mice. These recordings yielded equivalent results. Furthermore, validity was established when sensory threshold values remained unchanged with repeat testing that displayed identical side to side SE values in a wild type mouse. One operator allowed us to avoid interrater variability and we report our standards of error.

There was also a trend for higher amplitudes to be obtained from the right hind limb and longer latencies to be obtained from the left hind limb. These findings are not uncommon to electrodiagnosis. In the presence of side to side differences, reference values have previously been established. Mysiew¹⁹ and Foley²⁰ demonstrated side to side differences

in establishing reference values for median nerve. Kanakamedala's findings also showed side to side differences in establishing reference values for the ulnar nerve at the elbow.²¹ As seen in their reporting, the differences found in this study were not statistically significant.

In obtaining these values, a number of montages were trialed. Initially, we stimulated subdermally at the level of the sciatic notch, but this yielded a motor recording. We also placed micrograbbers (Biopac Systems) around the fourth and fifth hind limb digits to record responses.

This technique was abandoned because the micrograbbers wrapped too tightly around the digits and applied excessive pressure on the digital nerves.

CONCLUSION

While electrodiagnostic studies have been previously performed on different mouse models,^{1-3, 6, 22} to our knowledge, this study is the first reporting of mixed nerve action potential reference values for the murine sural nerve. These values are useful for future research in the analysis and comparison of murine neurological disease models.

Acknowledgments

The authors thank Daniel Moore, MD and James Wells, MD for input, guidance, and support.

Disclosures: Supported in part by NIH grant AG026630 and CA111891.

Abbreviations

CMAP Compound motor action potential

REFERENCES

1. Han JJ, Carter GT, Weiss MD, Shekar C, Kornegay JN. Using electromyography to assess function in humans and animal models of muscular dystrophy. *Physical Medicine & Rehabilitation Clinics of North America*. 2005; 16(4):981–997. [PubMed: 16214055]
2. Han JJ, Carter GT, Ra JJ, Abresch RT, Chamberlain JS, Robinson LR. Electromyographic studies in mdx and wild-type C57 mice. *Muscle Nerve*. 2006; 33(2):208–214. [PubMed: 16281275]
3. Carter GT, Longley KJ, Entrikin RK. Electromyographic and nerve conduction studies in the mdx mouse. *American Journal of Physical Medicine & Rehabilitation*. 1992; 71(1):2–5. [PubMed: 1739439]
4. Osuchowski MF, Remick DG, Teener JW. Normative data for mouse sciatic nerve conduction. AANEM Conference. 2006
5. Chalazonitis A, Peterson ER, Crain SM. Nerve growth factor regulates the action potential duration of mature sensory neurons. *Proc Natl Acad Sci U S A*. 1987; 84(1):289–293. [PubMed: 3467356]
6. George A, Bostock H. Multiple measures of axonal excitability in peripheral sensory nerves: An in vivo rat model. *Muscle Nerve*. 2007; 36(5):628–636. [PubMed: 17654556]
7. Pachner AR, Kantor FS. Nerve stimulation test in murine experimental autoimmune myasthenia gravis. *Ann Neurol*. 1982; 11(1):48–52. [PubMed: 6277236]
8. Makowska A, Panfil C, Ellrich J. Long-term potentiation of orofacial sensorimotor processing by noxious input from the semispinal neck muscle in mice. *Cephalalgia*. 2005; 25(2):109–116. [PubMed: 15658947]
9. Suckow, M.; Danneman, P.; Brayton, C. *The Laboratory Mouse*. CRC Press; 2001.
10. Popesko, P.; Horák, J.; Rajtová, VA. *Colour Atlas of the Anatomy of Small Laboratory Animals*. Saunders Book Company; 2002.

11. Pease, W.; Lew, H.; Johnson, E., editors. *Johnson's Practical Electromyography*. 4th ed.. Lippincott Williams & Wilkins; Philadelphia: 2007.
12. Amoiridis G, Schols L. Is the sural nerve a pure sensory nerve? *Muscle Nerve*. 2000; 23(12): 1895–1896. [PubMed: 11102918]
13. Amoiridis G, Schols L, Ameridis N, Przuntek H. Motor fibers in the sural nerve of humans. *Neurology*. 1997; 49(6):1725–1728. [PubMed: 9409380]
14. Cuddon PA, Kitchell RL, Johnson RD. Motor fibers in the canine distal caudal cutaneous sural nerve--dual innervation of the hind limb plantar muscles. *Anat Histol Embryol*. 1989; 18(4):366–373. [PubMed: 2624328]
15. Haupts MR, Amoiridis G. The sural nerve is not always a pure sensory nerve. *Muscle Nerve*. 2007; 35(6):812. [PubMed: 17415750]
16. Peyronnard JM, Charron L. Motor and sensory neurons of the rat sural nerve: A horseradish peroxidase study. *Muscle Nerve*. 1982; 5(8):654–660. [PubMed: 7155177]
17. Peyronnard JM, Charron L, Lavoie J, Messier JP. Differences in horseradish peroxidase labeling of sensory, motor and sympathetic neurons following chronic axotomy of the rat sural nerve. *Brain Res*. 1986; 364(1):137–150. [PubMed: 2418915]
18. Cuddon PA. Electrophysiology in neuromuscular disease. *Vet Clin North Am Small Anim Pract*. 2002; 32(1):31–62. [PubMed: 11785734]
19. Mysiew WJ, Colachis SC 3rd. The pronator syndrome. an evaluation of dynamic maneuvers for improving electrodiagnostic sensitivity. *American Journal of Physical Medicine & Rehabilitation*. 1991; 70(5):274–277. [PubMed: 1910654]
20. Foley BS, Roslonski ET, Buschbacher RM. Reference values for median nerve conduction to the pronator quadratus. *Archives of Physical Medicine and Rehabilitation*. 2006; 87(1):88–91. [PubMed: 16401444]
21. Kanakamedala RV, Simons DG, Porter RW, Zucker RS. Ulnar nerve entrapment at the elbow localized by short segment stimulation. *Archives of Physical Medicine & Rehabilitation*. 1988; 69(11):959–963. [PubMed: 3190420]
22. Pearson KG, Acharya H, Fouad K. A new electrode configuration for recording electromyographic activity in behaving mice. *Journal of Neuroscience Methods*. 2005; 148(1):36–42. [PubMed: 15908013]

Figure 1.
Depiction of antidromic technique using near nerve stimulation

Figure 2.
Signal averaged tracing from the cutaneous surae caudalis. 1 is onset latency, 2 is peak latency, and 1-3 is the duration of the mixed action potential.

Figure 3. Mean amplitude and mean latency measurements using side to side comparison. The measurements in the side to side comparisons were not significantly different.