Video Article

In Vivo Electrophysiological Measurement of Compound Muscle Action Potential from the Forelimbs in Mouse Models of Motor Neuron Degeneration

Eveliina Pollari[1,2], Robert Prior[1,2], Wim Robberecht[1,2,3], Philip Van Damme[1,2,3], Ludo Van Den Bosch[1,2]

[1]Department of Neurosciences, Experimental Neurology, KU Leuven - University of Leuven

[2]Center for Brain & Disease Research, Laboratory of Neurobiology, VIB

[3]Department of Neurology, University Hospitals Leuven

Correspondence to: Ludo Van Den Bosch at Ludo.Vandenbosch@kuleuven.vib.be

URL: https://www.jove.com/video/57741
DOI: doi:10.3791/57741

Keywords: Neuroscience, Issue 136, Compound muscle action potential, electromyography, forelimb, neuromuscular diseases, motor neuron, brachial plexus, sciatic nerve, electrophysiology, mouse

Date Published: 6/15/2018

Citation: Pollari, E., Prior, R., Robberecht, W., Van Damme, P., Van Den Bosch, L. *In Vivo* Electrophysiological Measurement of Compound Muscle Action Potential from the Forelimbs in Mouse Models of Motor Neuron Degeneration. *J. Vis. Exp.* (136), e57741, doi:10.3791/57741 (2018).

Abstract

Assessing the functionality of the nerve axon provides detailed information on the progression of neuromuscular disorders. Electrophysiological recordings provide a sensitive approach to measure nerve conduction in humans and rodent models. To broaden the technical possibilities for electromyography in mice, the measurement of compound muscle action potentials (CMAPs) from the brachial plexus nerve in the forelimb using needle electrodes is described here. CMAP recordings after stimulating the sciatic nerve in hindlimbs have been previously described. The newly introduced method here allows for the evaluation of the nerve conductivity at an additional site, and thus provides a more profound overview of the neuromuscular functionality. The technique provides information on both the relative number of functional axons and the myelination level. Thereby, this method can be applied to assess both axonal diseases as well as demyelinating conditions. This minimally invasive method does not require extraction of the nerve and therefore it is suitable for repeated measurements for longitudinal follow-up in the same animal. Similar recordings are performed in clinical setups to emphasize the translational relevance of the method.

Video Link

The video component of this article can be found at https://www.jove.com/video/57741/

Introduction

Electrophysiology is used as a diagnostic tool in neuromuscular disorders such as motor neuron disorders, plexopathies, neuropathies, neuromuscular junction disorders, and myopathies. In amyotrophic lateral sclerosis (ALS), in which primarily the motor neurons are affected, the axonal damage and muscle paralysis[1] are reflected in reduced CMAP amplitudes on nerve conduction studies (NCS). In Charcot-Marie-Tooth disease (CMT) both axonal degeneration and demyelination can be estimated in peripheral nerves using NCS[2]. This technique can be used for confirming the diagnosis as well as to evaluate the disease progression[3,4]. NCS enable the estimation of the axonal pathology, which is deduced from the magnitude of the action potential amplitude[5], and the extent of demyelination - which results in reduced conduction velocity, prolonged distal latencies, or conduction block[6].

CMAP measurement is a fast and sensitive method to evaluate nerve conduction both in humans and mice. Whereas in patients the NCS are performed routinely at various sites to record different nerves and muscles, in mice, the CMAP measurements are typically done only for the sciatic nerve to assess nerve functionality in the hindlimbs. However, in some animal studies it would be advantageous to record CMAP both in the fore- and hindlimbs, for example, to follow differential disease progression between fore- and hindlimbs in ALS mouse models.

Here, we introduce a method for recording CMAPs from the forelimbs of mice using needle electrodes. Additionally, we provide an approach to measure CMAPs from hindlimbs, likewise with needle electrodes. The measurement of CMAPs from hindlimbs with ring electrodes has been presented earlier[7,8]. The recording of CMAPs using needle electrodes is a rapid measuring method, it does not require shaving of the fur, and the procedure for measuring both hind- and forelimbs takes only 10 min per animal for an experienced researcher. Moreover, this minimally invasive approach is feasible for repeated measurements to allow longitudinal follow-up of multiple nerves in animals.

Protocol

All animals were housed under standard conditions according to the guidelines of the KU Leuven - University of Leuven and the associated European guidelines (European Union Directive 2010/63/EU for animal experiments). All animal experiments were approved by the local ethical committee of the KU Leuven.

1. Animal Preparation and Anesthesia

1. Induce anesthesia in the mouse with isoflurane/oxygen inhalation. Use 4% of isoflurane for the induction of the anesthesia and 2-3% for the maintenance at 2.5 L/min flow of oxygen. Adjust the isoflurane percentage for the maintenance of the anesthesia according to the condition of the mouse, *i.e.*, small and weak mice require less anesthetics. Confirm adequate anesthesia *e.g.*, by applying mild pressure to the hindlimb walking pad to check the absence of a pain withdrawal reflex.
2. Control the mouse body temperature using a thermostatic heating plate at 37 °C to prevent the decrease of body temperature during anesthesia.
3. Fit the mouse with the nosecone for maintenance of the anesthesia. Ensure that the animal has sufficient delivery of oxygen by checking that the nosecone does not block airways and that the animal is breathing steadily.
4. During the recording, monitor whether the mouse is sufficiently anesthetized by observing the breathing rate (approximately 1 Hz in anesthesia) and the absence of a withdrawal reflex on mild pressure. Increase the isoflurane concentration manually if the anesthesia is not deep enough.
5. After the measurements, leave the mouse to recover on the heating plate or in the warmth of an infrared lamp until it has regained sufficient consciousness to maintain sternal recumbency, for approximately 2-5 min. Do not leave the mouse unattended and in the company of other mice until it has fully recovered from anesthesia.

2. Measurement of CMAP in Hind- and Forelimbs

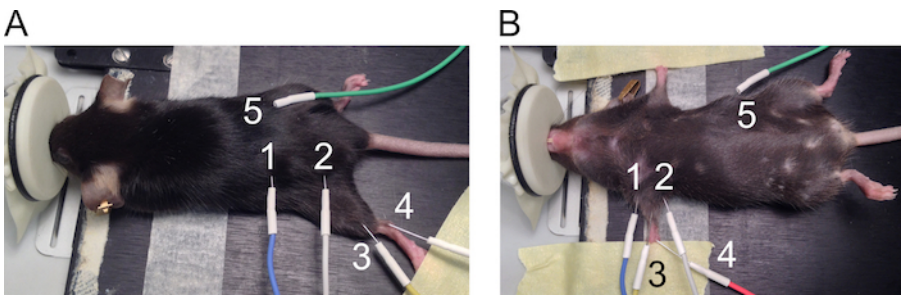

Figure 1. Positioning of the electrodes for CMAP measurements. The position of the electrodes is presented for hind- (**A**) and forelimbs (**B**). The electrodes are numbered as follows: 1: anode and 2: cathode stimulating electrodes, 3: active recording electrode, 4: reference electrode, and 5: grounding electrode. Please click here to view a larger version of this figure.

1. Use the 27 G needle electrodes for hindlimb and forelimb CMAP measurements. See **Figure 1** for recommended places of electrode positioning.
2. Place electrodes on the hindlimb as follows.
    1. Place the mouse on the heating pad in the prone position. Extend the hindlimb at the knee and attach the paw on the working surface using adhesive tape (**Figure 1A**).
    2. Place the stimulating electrodes subcutaneously on both sides of the sciatic notch with a distance of approximately 2 cm (1 = anode and 2 = cathode) between the electrodes. Lift the skin to insert the needle perpendicularly through the skin and push approximately 5 mm of the needle under the skin without puncturing the underlying muscles.
    3. Similarly, place the recording electrode (3) subcutaneously aligning the gastrocnemius muscle. Insert the reference electrode (4) subcutaneously next to the Achilles tendon in a 30-degree angle and leave 2-5 mm of the needle under the skin. Place the ground electrode (5) subcutaneously on the side of the mouse in a similar manner as the stimulating electrodes, but the position of this electrode is not critical for the measurement.
3. Place electrodes on the forelimbs as follows.
    1. Position the mouse on the heating pad in the supine position and use adhesive tape to extend both forelimbs on the sides of the body (**Figure 1B**).
    2. Place the stimulating electrodes (1 = anode and 2 = cathode) subcutaneously on both sides of the forelimb to align with the brachial plexus nerve. Lift the skin to insert the needle perpendicularly through the skin and push approximately 5 mm of the needle under the skin without puncturing the underlying muscles.
    3. Place the recording electrode (3) subcutaneously on top of the biceps brachii muscle by lifting the skin. Place the reference electrode (4) on the walking pads in 3 mm depth at a 30-degree angle. Place the ground electrode (5) subcutaneously on the side of the mouse. NOTE: Electrodes are in close proximity of each other in this setup. Prevent electrodes from touching each other as this distorts the recording.

3. Data Acquisition

1. Start the stimulation by pushing the **recurrent stimulus** button in the controller unit and turn the intensity controller knob to increase the stimulus. Stimulate all axons using 1 pulse/s with 0.1 ms stimulus duration. Select the correct frequency and duration from dropdown menus in the software.
2. To reach supramaximal stimuli (5-20 mA; in demyelinating conditions up to 60 mA), apply increasing stimuli by turning the intensity controller knob until the amplitude of the CMAP response ceases to increase. From there, further increase the stimulus by 20% to ensure that the CMAP amplitude has reached its maximal response. End the stimulation by pushing the **recurrent stimulus** button again.

3. Use the marker tool to indicate the following points in the recording: initiation of the stimulus, initiation of the response, maximum positive peak, and maximum negative peak (**Figure 2**).
4. Determine the latency (in ms) as a delay from the initiation of the stimulus to the initiation of the response (**Figure 2**). Define the initiation of the response as the earliest point where the amplitude begins to increase. Use the latency to evaluate demyelination in the axons.
5. Measure the amplitude (mV) from the maximum negative to maximum positive peak (**Figure 2**). Use the magnitude of the amplitude to correlate the number of functional axons.

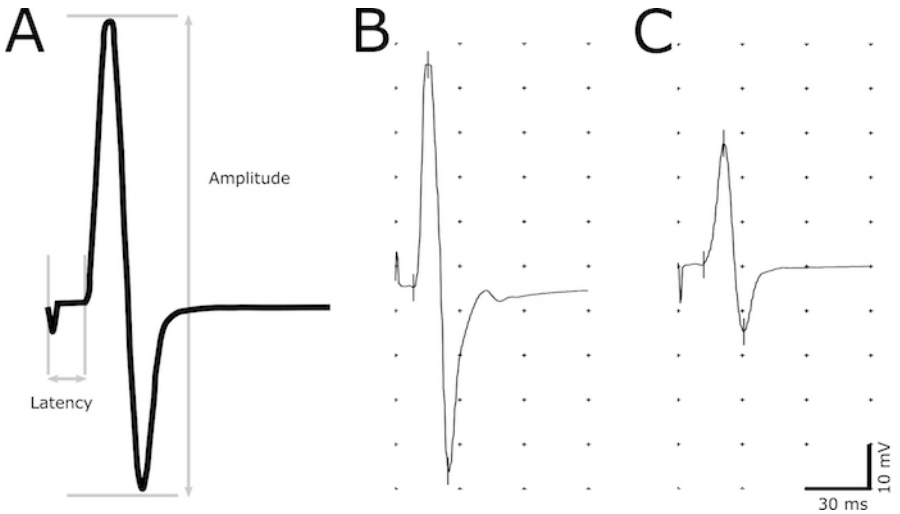

Figure 2. Representative image of CMAP response. A descriptive CMAP response indicating the points used for calculating the amplitude and latency (**A**). Latency is determined by the delay from the stimulation to the onset of the CMAP response. Peak-to-peak amplitude is measured from the maximum negative to the maximum positive peak of the biphasic wave. Representative recordings of a healthy non-transgenic animal (**B**) and a diseased animal with prolonged latency and reduced amplitude (**C**). Please click here to view a larger version of this figure.

6. Since the exact placement of the electrodes can affect the outcome value of the recording, replace the electrodes and measure the same nerve for three times using supramaximal stimulus to ensure that the largest response is obtained. Use the average of the recordings.

Representative Results

Electrophysiological measurements of CMAPs using needle electrodes is a minimally invasive and very sensitive method to follow neuromuscular function over time. The technique described here allows the assessment of forelimb nerve conduction in mice, and thus, provides insights into the functionality of the nerve.

The CMAP amplitudes and latencies were measured from hind- and forelimbs during disease course in two mouse models of ALS, SOD1-G93A[9] and PrP-hFUS-WT3[10] (**Figure 3**), and in a mouse model of CMT, C61-PMP22[11,12] (**Figure 4**). ALS mouse models were created by overexpression of ALS-related human genes, namely either mutated *SOD1* or wild type *FUS*. In both models, mice develop ALS resembling progressive motor neuron degeneration leading to paralysis. In non-transgenic littermate controls, the CMAP amplitude of both hind- and forelimbs did not change over time (**Figure 3A**). On the other hand, the CMAP amplitude of the sciatic nerve from the hindlimb was dramatically decreased in the SOD1-G93A mice, even before symptom onset around the age of 60 days (whereas the first motor symptoms are usually observed at the age of three months)[13]. The amplitude was 90 mV at that age in non-transgenic (non-tg) littermates, whereas in SOD1-G93A mice it was only 30 mV. There was only minimal further decline in the amplitude as the disease progressed to late symptomatic stage at the age of 150 days. The decline in CMAP amplitude, and hence the degeneration of axons, was delayed in the brachial plexus nerve of the forelimbs in comparison to the sciatic nerve from the hindlimbs. In the forelimbs, the disease progression was also more noticeable as the CMAP amplitude decreased from 70 mV to 30 mV when measured before and after the manifestation of the motor deficits in these mice.

In the PrP-hFUS-WT3 mouse model of ALS, the onset of motor deficits starts approximately at the age of 28 days[10], which coincides with the initiation of the decline in the CMAP amplitude. This is a more accelerated disease model as the mice reach end-stage approximately at the age of 65 days. The decline in the CMAP amplitude occurred more rapidly in the sciatic nerve of the hindlimb in comparison to the brachial plexus nerve in the forelimb, which indicates an earlier axonal degeneration in the hindlimbs (**Figure 3D**). This observation supports the clinical observation in both of these mouse models as the hindlimbs are paralyzed notably earlier than the forelimbs that remain functional up to the late stages of the disease process.

In general, the latency from stimulus to initiation of the action potential was shorter in the forelimbs compared to hindlimbs (**Figure 3B, E**). This is simply due to the shorter distance between the stimulating and the recording electrodes. The latency provides an indication of the myelination level of the axons. Our observation is that CMAP latencies are prolonged during the disease progression in mouse models of ALS, although ALS is not a demyelinating disease. This is most likely due to the loss of larger, faster conducting motor axons.

The C61-PMP22 mice overexpressing 3-4 copies of the human PMP22 and the heterozygote mice recapitulate a very mild CMT1A disease phenotype with mild demyelination and reduced CMAPs, but with no visible phenotype[11,12]. In 1.5-2 years of age C61-PMP22 mice, the CMAP amplitudes are reduced and latencies prolonged both in the hindlimbs and forelimbs (**Figure 4**). Representative recordings displaying diminished amplitude and delayed response in comparison to a recording from a healthy subject are presented in **Figure 2C, B**, respectively. The CMAP latencies in the forelimbs are not affected as much as in the hind limbs. This is consistent with CMT1A patients, as more often patients have severely reduced or undetectable CMAPs in the lower limbs due to the pathophysiological nature of CMT as a length-dependent disorder[14]. Additionally, the degree of disease severity is correlated with CMAP amplitude, rather than latency or conduction velocity, as amplitudes correlate with the degree of axonal integrity[14,15]. Nevertheless, results indicate that this method is sensitive enough to the detect demyelinating process such as those observed in CMT1A.

Variation in amplitude and latency was lowest in non-transgenic groups (coefficient of variation 2-15% and 1-13%, respectively). In all transgenic cases, there was more variation in the measurements (coefficient of variation for amplitude 8-51% and for latency 1-21%), which most likely is caused by the differences in disease progression among the animals. In all cases, the variation was similar in hind- and forelimbs. The variation in the use of needle and surface electrodes has been reported to be similar[16].

The required stimulus intensities did not vary greatly between non-transgenic and ALS models (**Figure 3C, F**). Likewise, the required stimulus to reach supramaximal stimulus in these cases was similar for fore- and hindlimbs and varied between 5-12 mA. In CMT, the requirement for increased stimulus intensities has been recognized[17] and the same phenotype was seen in C61-PMP22 mice (**Figure 4C**). The phenomenon has been explained by increased electrical impedance from hypertrophic endoneurial changes[17].

To confirm that the CMAP amplitude recorded from the forelimbs was due to nerve stimulation and not muscle stimulation, we performed unilateral partial axotomy on the brachial plexus nerve in 5 months old non-transgenic C57BL/6Jax mice (male and female) (**Figure 5**). Axotomy reduced the CMAP amplitude from 90 mV to 20 mV, indicating that most of the axons were disconnected in the operation. There was no change in the amplitude in the contralateral forelimb or in the hindlimbs. This result strongly indicates that the response detected in the biceps brachii was due to nerve stimulation and did not result from muscle stimulation.

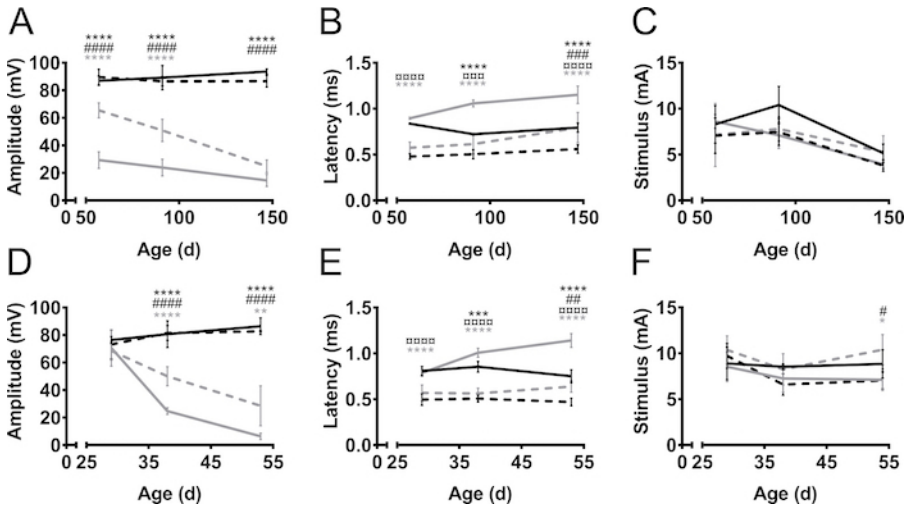

Figure 3. CMAP amplitude, latency, and required stimulus over the disease course in the hind- and forelimbs in ALS mouse models. SOD1-G93A (**A-C**) and PrP-hFUS-WT3 (**D-F**) transgenic (tg) mice and non-transgenic (non-tg) littermates were measured at the onset of the motor symptoms, at the symptomatic stage, and in the late-symptomatic phase of the disease process, at ages 57, 91, and 147 days (d) or at 29, 38, and 53 days for SOD1-G93A and PrP-hFUS-WT3 mice, respectively. Black: Non-transgenic hindlimb, black dashed: non-transgenic forelimb, grey: transgenic hindlimb, grey dashed: transgenic forelimb. Results are presented as mean ± SD. Amplitudes (**A**, **D**) were stable over time in the non-transgenic animals both in hind- and forelimbs. In transgenic animals, amplitudes decreased during the disease process. Latencies (**B**, **E**) were less affected by the disease and major differences were observed between hind- and forelimbs, regardless of the genotype. Variation in the required stimulus (**C**, **F**) was minimal in all groups. For SOD1-G93A N = 4 in all groups except for tg 147 d, N = 3. For PrP-hFUS-WT3 mice in age groups 29, 38, and 53, N is for non-tg 4, 5, and 4, and for tg 7, 5, and 3, respectively. Symbols denote the difference between groups as follows: *: non-tg hindlimb vs. tg hindlimb, #: non-tg forelimb vs. tg forelimb, ¤: non-tg hindlimb vs. non-tg forelimb, grey *: tg hindlimb vs. tg forelimb. Two-way ANOVA with Tukey's multiple comparisons test, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.#: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$, ####: $p < 0.0001$.¤: $p < 0.05$, ¤¤: $p < 0.01$, ¤¤¤: $p < 0.001$, ¤¤¤¤: $p < 0.0001$. Please click here to view a larger version of this figure.

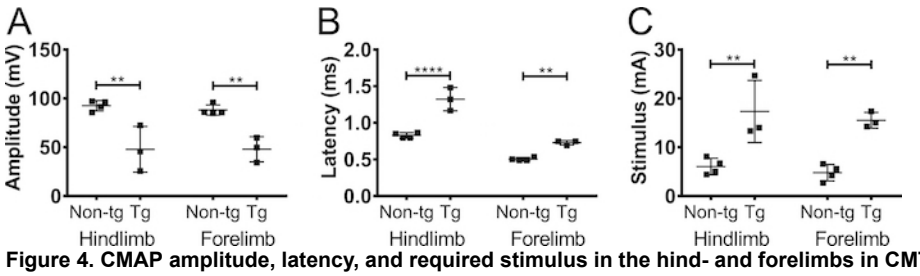

Figure 4. CMAP amplitude, latency, and required stimulus in the hind- and forelimbs in CMT1A mice. C61-PMP22 transgenic (tg) mice and non-transgenic (non-tg) littermates were measured at 1.5-2 years of age. Amplitude (**A**) was decreased both in hind- and forelimbs in transgenic mice. Latency (**B**) was prolonged in all limbs in CMT mice and even subtle change in forelimbs was detected with this measurement. Requirement for stimulus intensity (**C**) was increased in C61-PMP22 mice, which resembles the detected phenotype in CMT1A patients. Results are presented as mean ± SD, for non-tg N = 4 and tg N = 3. Two-way ANOVA with Sidak's multiple comparisons test,**: $p < 0.01$, ****: $p < 0.0001$. Please click here to view a larger version of this figure.

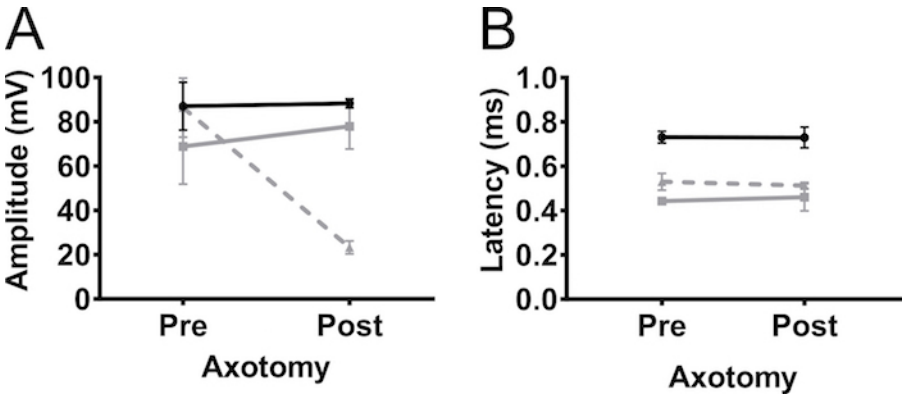

Figure 5. Forelimb action potentials are caused by nerve stimulation. To exclude the possibility that the observed CMAP response was caused by muscle stimulation, (partial) axotomy was performed on the brachial plexus nerve. CMAP amplitude (**A**) and latency (**B**) were recorded before (pre) and 4 days after (post) the axotomy of the brachial plexus in adult non-transgenic mice. Axotomy diminished the CMAP amplitude indicating that the response was due to nerve stimulation. Black: hindlimb, grey: contralateral forelimb, grey dashed: ipsilateral forelimb. Results are presented as mean ± SD, N = 2. Please click here to view a larger version of this figure.

Discussion

Sensitive recording methods are essential for assessing the disease progression and especially the efficacy of a therapy in animal models of neuronal disorders. Determining the CMAPs is a minimally invasive electrophysiological technique, which is routinely used in clinics and in experimental setups to assess nerve conduction in neuromuscular and neuropathic disorders[3,18]. Here, we describe a novel application for CMAP recording in mice in order to measure the nerve conduction in the brachial plexus nerve of the forelimb. The presented method allows a more versatile and detailed longitudinal assessment of neuronal function in mouse models of neurodegeneration.

The needle electrodes are slightly more invasive than ring electrodes and especially in longitudinal studies care must be taken to minimize tissue damage. One possible drawback of the method is resulting injury from piercing a nerve or muscle. However, after careful subcutaneous placement of the electrodes, the injury and disruption of the muscles and nerves can be prevented. In contrast to the method using ring electrodes, the method presented here does not require shaving of the fur from large parts of the body. As a consequence, there is no discomfort or effect on thermoregulation for the animal.

The positioning of the electrodes is critical for the correct and consistent recording of the CMAP amplitudes and latencies. It is advisable to reposition the electrodes and to perform two to three measurements at each site to confirm that the maximal stimulation and responses are achieved. Correct recordings should produce biphasic curves as demonstrated in **Figure 2**. To standardize the method, non-transgenic mice without nerve injury are the best models to establish the proper and consistent electrode positioning for optimal stimulation. Reusable needle electrodes are suitable for repeated use if they are regularly sterilized, e.g. with glutaraldehyde for 20 minutes between animals, and inspected for sharpness.

In healthy adult mice, the CMAP amplitudes recorded with the presented method are typically 80-100 mV after stimulating the sciatic nerve and the brachial plexus. This is notably larger than the responses measured with ring electrodes, because there is a higher impedance caused by the skin for the ring electrodes that produces results of 20-40 mV[8,19,20]. In ALS mouse models, the CMAP amplitudes after stimulating the sciatic nerve or the brachial plexus in paralyzed limbs decrease to 10-30 mV. The magnitude of the CMAP amplitude is smaller in young animals since the CMAP amplitude increases during development[21].

The method that we describe here is especially useful in mouse models of ALS, in which denervation, and subsequent motor deficits, occur earlier in the hindlimbs than in the forelimbs[13]. In addition to denervation, the method could detect reinnervation which is determined as prevented or retarded decline in the CMAP amplitude. The dramatic decrease in the CMAP amplitude in the muscles of the hindlimbs already at the age of symptom onset hinders the follow-up of further disease progression; as the CMAP amplitudes reach very low values at the early stage of the disease, they do not further decrease during the disease process. In contrast, axonal loss progresses at a slower pace in the brachial

plexus nerve of the forelimbs and presents a more sensitive option for measuring disease progression over a longer duration of the disease. Moreover, the less degenerated forelimbs could provide a more potent site for assessing therapeutic approaches that aim at enhancing axonal function.

It is clear that the presented technique provides novel possibilities for the characterization of mouse models of neuromuscular disorders. CMAP recordings with needle electrodes from the sciatic nerve and the brachial plexus is a rapid and reproducible method to assess axonal loss and demyelination in hind- as well as in forelimbs. The sensitivity of the method enables detection of axonal deficits even before notable motor deficits can be recorded, and thus allows the early quantification of these defects. Moreover, the possibility of repeated testing reduces the number of required animals and provides a detailed overview of the progression of neuromuscular and neuropathic diseases at different sites in an individual animal.

Disclosures

The authors have nothing to disclose.

Acknowledgements

This research has been supported by KU Leuven ('Opening the Future' and C1), the Fund for Scientific Research Flanders (FWO-Vlaanderen), the Thierry Latran Foundation, the Association Belge contre les Maladies neuro-Musculaires (ABMM), the Muscular Dystrophy Association (MDA), the and ALS Association and the ALS Liga (Belgium). PVD holds a senior investigatorship of FWO-Vlaanderen. RP was supported by grants from the Central Remedial Clinic (CRC) Ireland and is currently supported the National University of Ireland (NUI) and the FWO.

References

1. Brown, R. H., & Al-Chalabi, A. Amyotrophic Lateral Sclerosis. *N Engl J Med.* **377** (2), 162-172 (2017).
2. Prior, R., Van Helleputte, L., Benoy, V., & Van Den Bosch, L. Defective axonal transport: A common pathological mechanism in inherited and acquired peripheral neuropathies. *Neurobiol Dis.* **105** 300-320 (2017).
3. de Carvalho, M. *et al.* Electrodiagnostic criteria for diagnosis of ALS. *Clin Neurophysiol.* **119** (3), 497-503 (2008).
4. Krajewski, K. M. *et al.* Neurological dysfunction and axonal degeneration in Charcot-Marie-Tooth disease type 1A. *Brain.* **123 (Pt 7)** 1516-1527 (2000).
5. Raynor, E. M., Ross, M. H., Shefner, J. M., & Preston, D. C. Differentiation between axonal and demyelinating neuropathies: identical segments recorded from proximal and distal muscles. *Muscle Nerve.* **18** (4), 402-408 (1995).
6. Zielasek, J., Martini, R., & Toyka, K. V. Functional abnormalities in P0-deficient mice resemble human hereditary neuropathies linked to P0 gene mutations. *Muscle Nerve.* **19** (8), 946-952 (1996).
7. Arnold, W. D. *et al.* Electrophysiological Motor Unit Number Estimation (MUNE) Measuring Compound Muscle Action Potential (CMAP) in Mouse Hindlimb Muscles. *J Vis Exp.* (103) (2015).
8. Schulz, A., Walther, C., Morrison, H., & Bauer, R. *In vivo* electrophysiological measurements on mouse sciatic nerves. *J Vis Exp.* (86) (2014).
9. Gurney, M. E. *et al.* Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science.* **264** (5166), 1772-1775 (1994).
10. Mitchell, J. C. *et al.* Overexpression of human wild-type FUS causes progressive motor neuron degeneration in an age- and dose-dependent fashion. *Acta Neuropathol.* **125** (2), 273-288 (2013).
11. Robertson, A. M. *et al.* Comparison of a new pmp22 transgenic mouse line with other mouse models and human patients with CMT1A. *J Anat.* **200** (4), 377-390 (2002).
12. Huxley, C. *et al.* Correlation between varying levels of PMP22 expression and the degree of demyelination and reduction in nerve conduction velocity in transgenic mice. *Hum Mol Genet.* **7** (3), 449-458 (1998).
13. Turner, B. J., & Talbot, K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. *Prog Neurobiol.* **85** (1), 94-134 (2008).
14. Manganelli, F. *et al.* Nerve conduction velocity in CMT1A: what else can we tell? *Eur J Neurol.* **23** (10), 1566-1571 (2016).
15. Cornett, K. M. *et al.* Phenotypic Variability of Childhood Charcot-Marie-Tooth Disease. *JAMA Neurol.* **73** (6), 645-651 (2016).
16. Jacobson, W. C., Gabel, R. H., & Brand, R. A. Surface vs. fine-wire electrode ensemble-averaged signals during gait. *J Electromyogr Kinesiol.* **5** (1), 37-44 (1995).
17. Parker, V., Warman Chardon, J., Mills, J., Goldsmith, C., & Bourque, P. R. Supramaximal Stimulus Intensity as a Diagnostic Tool in Chronic Demyelinating Neuropathy. *Neurosci J.* **2016** 6796270 (2016).
18. Benoy, V. *et al.* Development of Improved HDAC6 Inhibitors as Pharmacological Therapy for Axonal Charcot-Marie-Tooth Disease. *Neurotherapeutics.* **14** (2), 417-428 (2017).
19. Xia, R. H., Yosef, N., & Ubogu, E. E. Dorsal caudal tail and sciatic motor nerve conduction studies in adult mice: technical aspects and normative data. *Muscle Nerve.* **41** (6), 850-856 (2010).
20. Srivastava, A. K. *et al.* Mutant HSPB1 overexpression in neurons is sufficient to cause age-related motor neuronopathy in mice. *Neurobiol Dis.* **47** (2), 163-173 (2012).
21. Arnold, W. D. *et al.* Electrophysiological Biomarkers in Spinal Muscular Atrophy: Preclinical Proof of Concept. *Ann Clin Transl Neurol.* **1** (1), 34-44 (2014).