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**Compound Muscle Activated Potential (CMAP) recordings from the triceps surae**

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1. **OBJECTIVE**

The objective of this SOP is to provide an experimental protocol to assess diagnosis and to monitor diseases of central and peripheral nervous system by recording action potentials from the muscle through an electrophysiological method, such as electromyography (EMG).

This technique is based on the fact that, once the action potential propagates along the axon to the muscle, the innervating motor units fire as a result of a cerebral stimulus. Then, the action potential induces the depolarization of the post-synaptic membrane of the neuromuscular junction (NMJ) and subsequently along the membrane of the muscle fiber. A motor neuron can activate hundreds of muscle fibers in synchrony with one single action potential, and the resulting electric signal is detectable from the muscle itself by EMG. When the appropriate nerve is stimulated electrically, the evoked responses can be measured with surface or needle electrodes at a distal muscle level. Compound Muscle Action Potential (CMAP) is the measure of the evoked potential.

Anterior horn motor neuron diseases, such as Spinal Muscular Atrophy (SMA), are characterized by muscle weakness, therefore the progression of the disease and the potential efficacy of therapies can be evaluated by means of EMG recordings. CMAP recordings have been performed in mice since many years and have the obvious advantage of performing a test which is also in current clinical use in humans.

2. **SCOPE AND APPLICABILITY**

This SOP will describe how to measure Compound Muscle Action Potential (CMAP) in the SMA type II mice (Jax n. 005025), or any other mouse model, by using electromyography (EMG) as a diagnostic method to evaluate the disease progression and therapies efficacy. Other parameters (i.e. MUNE, nerve conduction velocity, presence of fibrillation, SNAP) could be recorded by the same electromyography apparatus, but will not be discussed in the present document.

EMG recordings on SMA type II mice can be performed from post-natal day 10 on, as before this age the muscle size is too small to room the needle electrode. Also, before post-natal day 10, anesthesia induction with isoflurane is difficult due to the high respiratory frequency.

CMAP is a passive test to be performed on mice, and therefore the outcome of the measurements is operator-independent. The triceps surae is an elective muscle to be chosen as for its size and accessibility.

The following explanations are intended for researchers or technical personnel who have an established experience in working with small animals.
3. **CAUTIONS**

- **Electrical interference**
  - Any implanted electronic device in the animal can interfere with EMG recordings
  - Metal tables can interfere with EMG recordings
  - Connected electrodes or other conductive parts should not be in contact

- **Mice must be kept under isoflurane anesthesia while performing electromyographic recordings.**

- **Age and sex**
  - CMAP values will vary with development until reaching the adulthood
  - Males have higher CMAP values than females (groups should be sex-balanced)

- **Ongoing infectious disease in the mouse**
  - Protective gloves should be worn as some bleeding may occur after removing the needle electrodes

- **Avoid transthoracic or prolonged stimulation**

- **Variability in recordings performed by different operators.**

- **Bleeding tendency (e.g. caused by drug treatment)**
  - Surface electrodes rather than needle electrodes should be used

4. **MATERIALS**

   An initial, relatively expensive, equipment is necessary. Following costs are due to maintenance and replacement of needle electrodes after long-term usage.

**Electromyography (EMG) unit:**

- **Key point** main unit (AB 9033A007401, Alpine Biomed). Keypoint Portable System (220V International)

- **Electrode kit** (AB 9013S0164, Alpine Biomed)

- **Keypoint software** (AB 9033S0511, Alpine Biomed)

**Anesthetic induction (Isoflurane anesthesia system):**

- **O₂ cylinder**
- **Induction chamber**
C. surgical table with anesthesia mask
D. isoflurane

5. METHODS

1. Start computer and software. Set the magnitude and time of stimulation at 12.6mA and 0.1ms (set the curve display on the computer screen at 20mV and 2ms).
2. Place the mouse in the induction chamber and anaesthetize with isoflurane.
3. Remove the mouse from the induction chamber and place on the surgery platform with the nose of the rodent in the anesthesia mask. In case of need of adapting the device to a very small SMA mouse (post-natal day 10), cut out a finger from a plastic glove and use it to cover the entrance of the anesthesia mask. Perform a small incision in the plastic cover in order to conveniently place the face of the mouse inside the hole.
4. Fix the paws to the table with a piece of tape with the paw-palm facing up.
5. Insert the needle electrodes into the muscle. Unless specific reasons (i.e. unilateral viral administration into the hindlimbs), recordings can be performed on only one of the hindlimbs assuming nerve conduction would be the same on the two sides. As in fig.1, five different electrodes have to be correctly placed in different sites (the sub sequential order is not relevant):

Fig. 1: Needle electrodes placement during EMG recordings. The mouse is kept under isoflurane anesthesia.
- The ground needle electrode (E1) has to be placed into the belly of the opposite gastrocnemius muscle.

- The reference needle electrode (anode-E2) should be inserted near the Achille’s tendon, the recording electrode (cautode-E3) is inserted into the middle of the gastrocnemius.

- An anode needle electrode (E4) is subcutaneously inserted at the tail base, the stimulus needle electrode (cautode-E5) is superficially placed on top of the skin at a paraspinal site.

6. Place the stimulus needle electrode (E5) at a paraspinal site ensuring the stimulation of the sciatic nerve, i.e. before the lumbar region. All the motor units between the stimulus and the reference electrode at the tail base will fire. The evoked CMAP response measured will account only for the firing of the sciatic nerve, which innervates the gastrocnemius muscle.

7. To obtain the CMAP response, apply the current by pressing the button on the electromyographic apparatus and perform two different stimulations (to be sure that the stimulus needle electrode (E5) was correctly placed) without changing the recording needle electrode (E3) position into the muscle.

8. Remove the recording needle electrode (E3) from the muscle and place it into another site of the muscle belly. Record another pair of responses. Because skeletal muscles differ in the inner structure, the electrode has to be placed at various locations to obtain an accurate study.

9. Repeat points 6 to 8.

10. Disconnect the electrodes and place the mouse back in the cage.

11. Recover from anesthesia will occur with spontaneous clearance of the isoflurane from the respiratory system and normally within few minutes.

6. EVALUATION AND INTERPRETATION OF RESULTS

Nerve conduction studies are performed at a distal muscle in order to record a pure motor neuron, not sensory, stimulation. Following stimulation, the CMAP evoked responses of a treated or untreated SMA mouse can be compared with untreated SMA mice (until survival limit) or non-affected littermates.

A typical CMAP curve will appear as in Fig. 2. Once the stimulus is applied to the threshold level, the action potential will initiate with the Na\(^+\) channels opening (depolarization phase). Then, K\(^+\) channels will open and repolarize the cells (repolarization phase). The peak amplitude is the area under the curve (about +80mV in a control mouse; second column of data in the picture) and is a measure of the activity of the motor units.
Fig.2 Typical CMAP results following electrical stimulation of the gastrocnemius muscle in an adult mouse.

EMG recordings can also help distinguish between different disease groups. Indeed in humans, anterior horn cells diseases, such as SMA, would have smaller CMAP compared to healthy individuals. Also, CMAP is an objective outcome measure to correlate motor function to the disease severity being able to discriminate between SMA type-II or -III patients (Lewelt et al., 2010). CMAP amplitudes will also be low in a primary muscle disease where a diminished number of muscle fibers are present. On the contrary conduction velocity would not be affected, as the remaining motor neurons, which are responsible for the obtained CMAP, will have a normal nerve conduction velocity. The case of a slow nerve conduction velocity is indeed observed in demyelinating nerve disease, which will display normal CMAP amplitudes. Repetitive nerve stimuli at a short interval could also show a neuromuscular junction disease, as decremental CMAP values will be observed.
7. REFERENCES


