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## Synaptic transmission alterations in SMA mice/ Electrophysiology

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SMA\_M.1.2.001

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**TABLE OF CONTENTS**

OBJECTIVE.....	3
SCOPE AND APPLICABILITY.....	3
CAUTIONS.....	3
MATERIALS.....	3
METHODS.....	4
EVALUATION AND INTERPRETATION OF RESULTS.....	5
REFERENCES.....	6
APPENDIX.....	7

## **1. OBJECTIVE**

The objective of this SOP is to provide the main guidelines for performing electrophysiological recordings of neurotransmission in neuromuscular junctions (NMJs). We describe the procedures for intracellular recording of evoked and spontaneous miniature end-plate potentials in skeletal muscle cells.

## **2. SCOPE AND APPLICABILITY**

SMA mice present a defective motility phenotype. The electrophysiological techniques can be particularly useful for investigating neuromuscular transmission in different types of skeletal muscles, and at different ages. Intracellular recordings in muscle fibers can be performed from neonatal mice to adults, and information about possible developmental defects during NMJ development can be obtained. It can also be used for investigating the pathogenic mechanisms involved in this disease. Finally, it can be used to assess the degree of recovery of the nerve terminals function in response to a particular drug treatment.

## **3. CAUTIONS**

The neuromuscular preparation need to be kept under physiological conditions. Damage to the tissue may result in changes to the electrical properties of nerve and muscle cells, such as low resting membrane potential. Therefore, recordings from fibers with low or unstable membrane potentials should be discarded.

A good practice is to compare results from wild-type and mutants mice from the same litter to minimized variations due to external factors.

## **4. MATERIALS**

The electrophysiological set-up for intracellular recording of muscular electrical activity requires the following equipment and instruments:

- upright microscope (Olympus, Nikon, Zeiss, etc)
- anti-vibration table (Newport, TMC, etc)
- Faraday cage
- micromanipulators (Narishige, Newport, etc)
- current clamp, or voltage clamp amplifier (Neurodata, NPI, etc)
- isolated pulse stimulator (AM-Systems, Warner Instruments, etc)
- analog-to-digital converter interface (ADInstruments, National Instruments, etc)
- pipette puller (Sutter Instruments, Kopf, etc)
- dissecting microscope (Nikon, Olympus, Mantis, etc)

Supplies:

- thin wall glass capillaries with filament (AM-Systems) for electrode fabrication

- dissecting tools (FST, etc)
- insect pins (FST)
- recoding chamber
- silicon elastomer (Dow Corning: Sylgard)
- suction electrode (home-made or commercial)
- chemicals (Sigma, Fluka, Alomone, etc)
- gravity fed perfusion system (solution containers, polyethylene tubes, etc)
- silver wire
- clay

## 5. METHODS

### **A. Solutions**

The Ringer solution used for the dissection and recording could have the following composition (in mM): 125 NaCl, 5 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 25 NaHCO<sub>3</sub>, 1 NaH<sub>2</sub>PO<sub>4</sub>, and 15 glucose. The solution should continuously be gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>, which maintained the pH at 7.35. The osmolarity of each solution may be adjusted to 300-330 mosmol/L. The solution should be gassed before adding the CaCl<sub>2</sub> to prevent the formation of precipitates.

### **B. Neuromuscular preparation**

The muscle of interest need to be dissected with its nerve supply intact and pinned to the bottom of a small Sylgard (Dow Corning Corp., USA) -lined chamber with with fine insect pins at approximate resting length. Preparations should be continuously perfused with a Ringer solution (see above). The preparation should be incubated for at least 30 min in Ringer solution before starting the recording.

### **C. Micropipette fabrication**

For end-plate potential measurements, intracellular electrodes are pulled to obtain pipettes with a long taper having resistance of 15-25 MΩ when filled with the conducting solution. For voltage clamp, lower resistance electrodes (5-15 MΩ) are better as they are lower noise.

### **D. Intracellular recording**

The neuromuscular preparation pinned to the bottom of the recording chamber is transferred to the stage of the up-right microscope. The bathing recording solution could have the same composition as the dissecting Ringer solution.

The ground electrode (a silver chloride wire) should connect the recording bathing solution to the headstage ground.

The nerve can be stimulated either by means of a suction electrode (Figure 1) or by a bipolar electrode (FHC, Bowdoin, ME). While suction electrodes are fine, they are a little hard to make as they have to be exactly the right size for the nerve. The bipolar electrode can easily

be placed on the nerve. The stimulation consists of square-wave pulses of 0.2-0.5 ms duration and 2–40 V amplitude, at variable frequencies (0.5–20 Hz).

Muscular contraction can be prevented by including in the bath 1–2  $\mu\text{M}$   $\mu$ -conotoxin GIIIB (Alomone Laboratories, Israel), a specific blocker of muscular voltage gated sodium channels. If one is using voltage clamp (Kong et al., 2009) one can also prevent muscle contraction by crushing the muscle fibers away from the endplate band. This depolarizes the fibers, inactivates sodium channels and thus prevents contraction. There is an art to this as the crush distance from the endplates needs to be just right. Once the technique is working, however, it is much cheaper than using conotoxin.

The glass microelectrode (10–25  $\text{M}\Omega$ ) is filled with 3 M KCl, or potassium acetate and this solution is connected via an Ag/AgCl electrode to the headstage of the amplifier and from there to the rest of the amplifier. Once the glass microelectrode is in the bath recording solution, electrode tip potential need to be adjusted and the input capacitance and electrode resistance neutralized.

By means of a three axis micromanipulator the microelectrode is positioned near a nerve terminal ending and the muscle fiber impaled (Figure 2). Alternatively one can put two electrodes into the fiber and voltage clamp it. While this is more difficult, it has the advantage that muscle fiber size, and its effect on input resistance, will have no effect on the recording.

Evoked endplate potentials (EPPs) and miniature EPPs (mEPPs) can be recorded from different NMJs within the muscle as described previously (Ruiz et al., 2010). The resultant analog signals can be sampled with an analog-to-digital converter at 10 KHz. The acquisition can be controlled by the software.

## 6. EVALUATION AND INTERPRETATION OF RESULTS

The mean amplitudes of the EPP and mEPPs recorded at each NMJ are normally linearly normalized to a resting membrane potential of  $-70$  mV. EPP amplitudes are corrected for nonlinear summation (McLachlan and Martin, 1981) as follows:

$\text{EPPc} = \text{Average Peak EPP} / (1 - 0.8 * \text{Average Peak EPP} / (V_m - E_r))$ , where  $V_m$  is the resting membrane potential and  $E_r$  the reverse potential (assumed to be between 0 and  $-5$  mV). This can all be skipped if one is using voltage clamp.

The kinetics of EPP and mEPP are characterized by their rise time (10–90%) and decay time constant (calculated from the exponential fit of the decay phase).

Quantal content (QC) can be estimated by the direct method, which consists of recording mEPPs and EPPs (nerve stimulation 0.5 Hz) simultaneously and then calculating the ratio:

$$\text{QC} = \text{Average Peak EPP} / \text{Average Peak mEPP}.$$

All experiments should include the results of at least three animals per genotype. Statistical comparisons between mutant and wild-type measures should be made using Student's t test

(two-tailed) for normal distribution and Mann–Whitney rank sum test when the distribution was not normal. Usually, statistical differences between wild type and mutants is considered significant at  $P < 0.05$ .

Typically, in SMN $\Delta$ 7 SMA motor terminals neurotransmission is impaired (Kong et al., 2009; Ruiz et al., 2010). In proximal muscles, the size of the evoked end-plate potentials (EPPs) is much decreased (see example in Figure 3). In these muscles the quantum content is to about half of that of wild-type littermates.

## **7. REFERENCES**

Kong L, Wang X, Choe DW, Polley M, Burnett BG, Bosch-Marcé M, Griffin JW, Rich MM, Sumner CJ. (2009) Impaired synaptic vesicle release and immaturity of neuromuscular junctions in spinal muscular atrophy mice. *J Neurosci.* 29(3):842-51.

McLachlan EM, Martin AR. (1981) Non-linear summation of end-plate potentials in the frog and mouse. *J Physiol.* 311:307-24.

Ruiz R, Casañas JJ, Torres-Benito L, Cano R, Tabares L. (2010) Altered intracellular  $Ca^{2+}$  homeostasis in nerve terminals of severe spinal muscular atrophy mice. *J Neurosci.* 30(3):849-57

8. APPENDIX

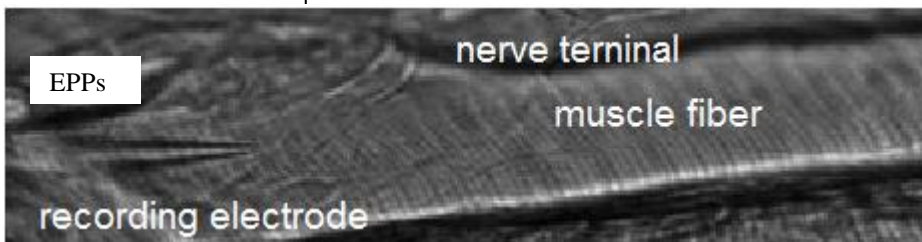
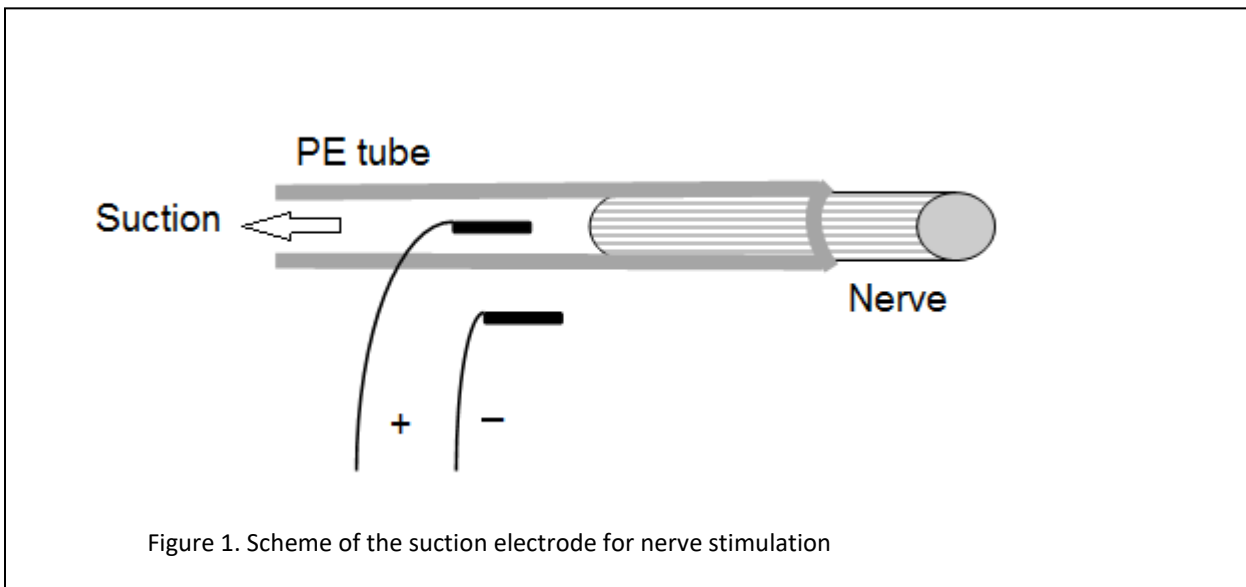


Figure 2. Microscopic view of the neuromuscular preparation during the electrical recording

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EPP

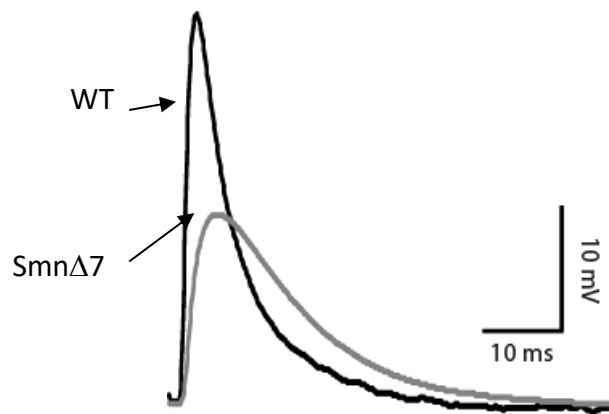


Figure 3: Representative examples of end-plate potentials from WT and Smn $\Delta$ 7 terminals from the TVA muscle. Note the smaller amplitude and slower kinetics of the signal in SMA.