

STANDARDIZATION OF MOTOR EVOKED POTENTIAL CAPTIVATION TECHNIQUE IN RATS THROUGH TRANSCRANIAL ELECTRIC STIMULUS

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SUMMARY

Experimentally, the authors demonstrate the standardization of the technique for capturing the motor evoked potential in rats through transcranial electric stimulus. Fifty Wistar rats, under anesthesia, were prepared according to the current rules of the Laboratory for Studies on Spinal Cord Trauma and Peripheral Nerves of the Orthopaedics and Traumatology Institute, Hospital das Clínicas, Medical School of São Paulo. Average minimal latency of the upper limb responses was 2.5 ms and of the lower limb 6.5 ms. Average

amplitude of the responses was 3.0 mV and 2.5 mV on upper and lower limbs, respectively. The authors conclude that the technique for obtaining motor evoked potential in rats, as presented in this study, is efficient for the analysis of the electrophysiological evolution of spinal cord injuries, unprecedented in our field, and can be reproduced in a simple way, presenting quality and applicability standards similar to those seen in global literature.

Keywords: Spinal cord Injuries; Wistar Rats; Electric stimulation.

INTRODUCTION

The search for appropriate knowledge on the mechanisms involved in spinal cord injuries has been strengthened in the last few years, justifying the increasingly production of experimental studies in the area of spinal cord regeneration⁽¹⁾.

Rats have been the animals mostly used in experimental models of spinal cord injuries⁽²⁾, maybe due to the ease for obtaining and dealing with this kind of animal, as well as to the lower expenditures in research- intended financial resources.

Functional recovery after spinal cord injury in rats may present different functional patterns depending on the severity of trauma and on the treatment given. The record of this recovery may be done through various behavioral tests in those animals^(3,4,5,6), anatomicopathological studies of the affected spinal cord segment^(7,8) and electrophysiological methods^(2,7,9,10,11,12).

There is no report in local literature concerning the study on the motor evoked potential in rats. To date, the evaluation of spinal cord injuries recovery in rats described in our environment has been performed only through clinical tests on motor function and histological tests^(13,14).

According to literature⁽²⁾, many electrophysiological methods have been developed for monitoring neurological recovery in rats. Some authors used stimulating screws directly attached to the skull, which remained inserted up to the end of the experimental study. In other studies, stimulating microelectrodes have been placed, directly attached to cerebral cortex; such procedures are technically different and more complex when compared to the transcranial stimulus model proposed in this study, of which needle electrodes are only inserted into animals' scalp.

OBJECTIVES

The objective of this study is to demonstrate the technique for obtaining motor evoked potential in rats, through transcranial

electrical stimulus, in a simple, economic and highly reproducible way for any experimental research laboratory.

MATERIALS AND METHODS

This study has been developed at the Laboratory for Studies on Spinal Cord Trauma and Peripheral Nerves (LETRAN) and at the Laboratory of Microsurgery of the Orthopaedics and Traumatology Institute - Hospital das Clínicas - Medical School, University of São Paulo (IOT - FMUSP), since 06/30/2003. Fifty male Wistar rats weighing 360g in average, and with average body temperature of 28° C, duly anesthetized with 55 – 75 mg/ kg of intraperitoneal Pentobarbital, associated to 55 – 75 mg/kg of intramuscular Ketamina.

Materials required for the procedure of obtaining motor evoked potential consist of a 4-channel electromyography instrument, 2 needle monopolar electrodes corkscrew EO401 type from Neuromedical Supplies for transcranial stimulation, 1 needle monopolar electrode to be used as grounding and four pairs of needle monopolar electrodes to be used on the capture of motor responses in UULL and LLLL.

Below, the steps for obtaining the motor evoked potential in rats will be described. Rats' weight (Figure 1A) is used both for calculating anesthetic dosage to be applied and for maintaining weight uniformity of the animals used in the experiment. Anesthesia (Figure 1B) intraperitoneal, with 55 - 75 mg/Kg of intraperitoneal Pentobarbital associated to 55 - 75 mg/Kg of intramuscular Ketamina. After animals were anesthetized, Trichotomy (Figure 1C) of the skull region was performed, in order to enable the insertion of needle electrodes into the scalp. Skull-tail length (Figure 1D) of the animal was measured between the occipital region and the point where tail basis begins. This datum shall be used for comparing latencies seen in different rats. Rats' temperature (Figure 1E) is measured on lower limb with a digital thermometer. The capture (Figure 1F

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- Figure 3) of muscular responses is performed by placing pairs of needle monopolar electrodes (capturer and reference), with determined and fixed interelectrodes distance for UULL and LLLL capture, inserted on proximal and anterior musculature of the UULL and LLLL. Grounding (Figure 3) electrode is placed on lumbar region through a needle monopolar electrode. Transcranial electric stimulation (Figure 2 and Figure 3) is performed by placing two corkscrew-type needle electrodes into the scalp on frontal region (anode) and occipital region (cathode) at the interhemispheric line, for simultaneous bilateral stimulus. After electrodes are placed in the rats, the equipment (Figure 4A) is turned on and the impedance (Figure 4B) of those electrodes is checked in order to prove their good adaptability so as to obtain

sharper, safer and reliable responses. Equipment calibration is performed in two aspects of muscular responses capture: scan: 20ms- window, sensitiveness: 2 mV/div., low-frequency filter: 10 Hz and high frequency filter: 10 KHz and the transcranial electric stimulation through a single stimulus, 0.2ms long. Stimulus intensity considered as supramaximal.

RESULTS

On Figure 5, some records of the motor evoked potential in rats are shown. Minimal latency measured on UULL responses was 2.5ms and on LLLL was 6.5ms. UULL responses average amplitude was 3.0mV and LLLL responses average amplitude was 2.5mV.

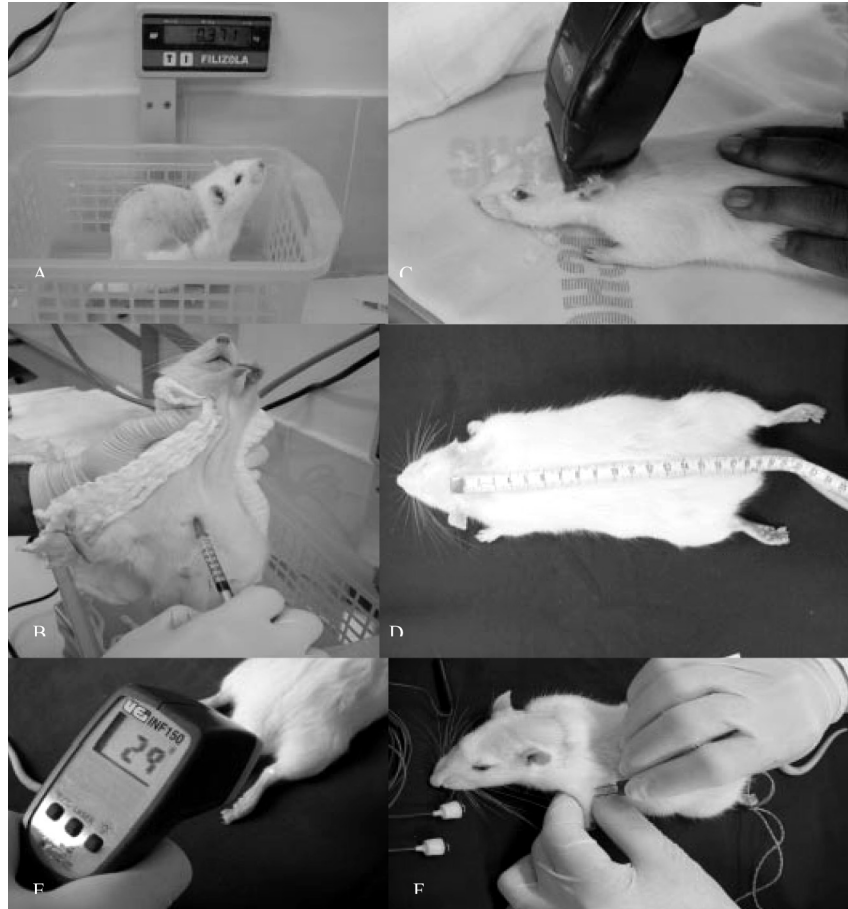


Figure 1 - A) Weight B) Anesthesia C) Trichotomy D) Measurement E) Temperature Measurement F) Fixation of a pair of needle electrode in MSE

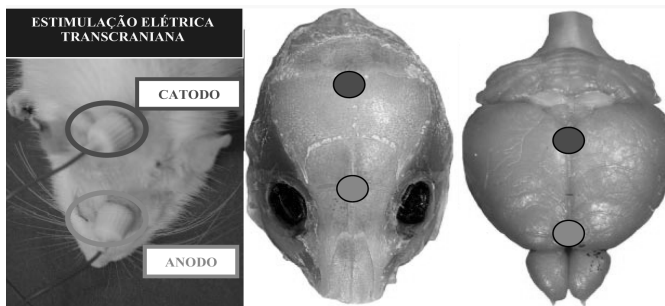


Figure 2 - Anode and Cathode stimulating electrodes

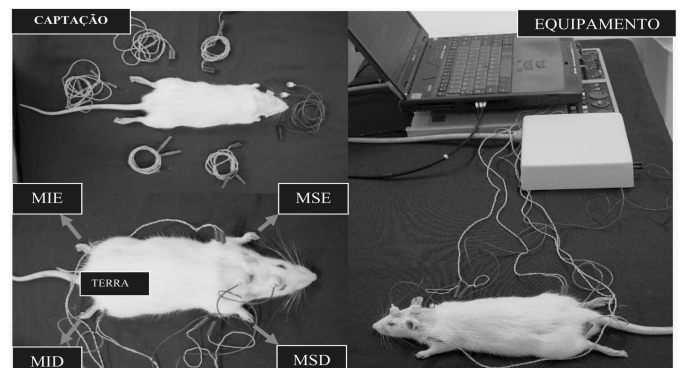


Figure 3 - Electrodes arrangement



Figure 4 - A) Equipment B) Impedanciometer

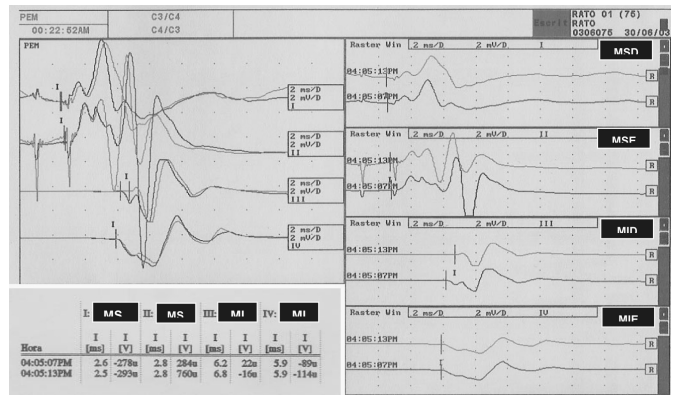


Figure 5 - Records of MEP motor responses

DISCUSSION

At the current knowledge level about spinal cord injury recovery, experimental studies are crucial to understand the mechanisms involved in injuries genesis, as well as to obtain functional improvement after spinal cord trauma. This kind of trauma is usually permanent and may cause devastating sequels for the individual, which reflects in family life and in the society in which he/ she exists.

In our environment, the study of neurological recovery in rats after spinal cord injury can also be evaluated with the motor evoked potential, in addition to clinical and histological tests that have already been proven in previous studies^(13,14).

The method presented in this study makes the achievement of

motor evoked potential in rates simpler. In literature, we did not find studies detailing existent techniques in a step-by-step fashion. Thus, we justify the conduction of this study, and also describe a feasible new evaluation method for neurological function in the increasing number of experiments performed in our environment.

CONCLUSIONS

We believe that the technique for capturing the motor evoked potential in rats as presented in this study is a safe method for analyzing the electrophysiological evolution of the spinal cord injury, and can be reproduced in a simple way, in any research laboratory, in addition to present quality and applicability standards similar to those seen in worldwide literature.

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