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Original Article

Recording of motor and somatosensory evoked potential in an anaesthetised Wistar rat using digital polyrite system

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ABSTRACT

Objectives: The aim of this article is to explain the detailed methodology to record Motor evoked potential (MEP) and somatosensory evoked potential (SSEP) in adult albino Wistar rat, male (200–250 g) which has not been defined previously.

Materials and Methods: We have standardised recording of both MEP and SSEP in these rats under anaesthesia on ADI digital polyrite system.

Results: Evoked potentials have been widely studied in spinal cord injured patients to estimate the degree of injury and to establish a predictive measure of functional recovery. MEPs and SSEPs, arising from the motor cortex or peripheral nerve and generated either by direct electrical stimulation or by transcranial magnetic stimulation, have been advocated as a reliable indicator of descending and ascending pathway integrity. In the rat brain, there is a physical overlap between the motor and somatosensory cortex. Hence, our objective was to identify the exact area for stimulation in the cortex where we could record maximum response with the application of minimum electrical stimulation.

Conclusion: The recording of MEP and SSEP together provides a powerful neurological technique to monitor the tracts of the spinal cord.

Keywords: Corticospinal tracts, Motor evoked potential, Sensory-motor cortex, Somatosensory evoked potential, Wistar rats

INTRODUCTION

The evoked potential has been widely used for evaluating motor and sensory systems clinically and experimentally in different species. It has been used as a quantifiable parameter for the functional assessment of spinal tracts. Evoked potential response is a direct electrophysiological method to measure the integrity of the neuronal network between the spinal cord and brain.[1,2] It is a very efficient parameter for assessing the extent of damage to ascending and descending tracts in variousneurodegenerative and neurotraumatic disorders such as Parkinson's disorder and spinal cord injury, respectively.[3-5] Various body parts are topographically represented in the motor and sensory cortex. Those areas regulate the movements and sensory perception of the corresponding peripheral areas.[6] Motor evoked potential (MEP) is defined as the response generated by the peripheral muscle/nerve when the corresponding brain area is stimulated

externally by mechanical, electrical, or magnetic stimuli. On the other hand, somatosensory evoked potential (SSEP) is the potential generated in the specific area of the somatosensory cortex of the brain when the corresponding peripheral nerve or muscle is stimulated. As any disruption or impairment to the motor or sensory tracts will affect the characteristics of the evoked potential, it becomes pertinent to record evoked potential precisely and accurately.

Multiple reports in various species and different disease models using MEP and SSEP[1-3,7] are available in the literature but none describes theprecise methodology of recording MEP and SSEP in Wistar male rats. In the present study, we intended to provide a detailed procedure forrecording MEP and SSEP in adult Wistar male rats under anaesthesia. Further, there are several crucial factors involved in the recording of evoked potential in an experimental setup which are important to get specific and reliable recordings. Level of anaesthesia, making of the window in the skull to the integrity of dura mater, and placement of electrodes could be the confounding factors. The use of multiple electrical systems can lead to the generation of significant electrical noise while recording MEP and SSEP. We have adopted various measures to improve our signal-to-noise ratio in this study. We have tried to minimise all the factors which can disrupt our recording.

MATERIALS AND METHODS

Animal details

Adult male Wistar rats weighing around 200–250 g procured from the central animal facility, AIIMS, New Delhi, were used for the present study. Each rat was housed individually in polypropylene cages (50 cm × 20 cm × 15 cm) and provided with laboratory food pellets (Ashirwad Industries, Ropar, Haryana, India) and clean drinking water *ad libitum*. The cages were cleaned every day and standard bedding was provided. Animals were housed and maintained at an ambient room temperature of 24±2°C, relative humidity of 50–55%, and the light-dark cycle of 14:10 h.

Animal ethics

The Institutional Animal Ethics Committee (File No. 781/IAEC/14) approved all procedures performed in the study. Care was taken to treat them humanely.

Experimental tools

Stereotaxy (Model-1404, David Kopf, USA)

We have used stereotaxy to follow a 3D Cartesian coordination system to target the specific brain area from Paxinos atlas.[8]

Micro-drill (Foredom Micro Drill, USA)

Micro-drill has been used to do the craniectomy, open the skull and expose the sensory-motor cortex.

Electrophysiology stimulation recording unit

It consists of a stimulation unit, isolator, and amplifier. We have used stainless steel and silver needle electrode and bipolar concentric electrodes for stimulating and recording various electrophysiological parameters.

ML1001 electronic stimulator

For electrophysiological stimulation, electrodes were connected with the stimulator unit (Nihon Kohden, Japan). Various stimulation parameters such as frequency, duration, and the interval between pulses can be modulated with this stimulator.

ML1101 stimulus isolator

The output from the electronic stimulator was fed to an isolator (ADI, ML1101). It has an output monitor, which shows the amount of current passed through the stimulating electrodes. It also has a fine adjustment knob to modulate the strength of current from 0.1 μA to 11.2 μA range. The output of the isolator was fed to the stimulation electrodes connected to the specific part of the rat muscle or nerves.

Animal bio amp FE136

This is a galvanically isolated, high-performance and software-controlled differential amplifier suitable for the measurement of a wide variety of biological signals in animals and isolated tissues. This amplifier unit has three ports for one positive, one negative, and one reference electrode. This amplifier unit is connected to the recording electrodes.

ML826 PowerLab

This is an eight analog input channel containing a power lab, four of which can be used in differential mode. It is16-bit with a 400kS/s ADC giving a maximum per channel sampling rate of 200 kS/s. The PowerLab is connected with an isolator, bio amplifier as well a digital monitor.

Electrodes

Needle electrodes

We have used an AD instruments Pvt Ltd stainless steel needle electrode for stimulation of the motor cortex (Model no- MLA1213; 29 ga, 1.5 mm Socket).

Hook electrode

We used micro hook electrodes for the recording of MEP and stimulation of the sciatic nerve for generating SSEP (Model no- **MLA1212;** Micro-Hook Electrodes, 1.5 mm Socket).

Concentric electrode

We fabricated a concentric electrode by the amalgamation of two stainless steel needles and insulated the electrode. We have inserted one insulated 27G, 1-inch needle into an 18G needle and connected them with respective wires. In the end, we soldered these two needles, blunt the tips of the needle, and insulated the whole setup. The outer diameter of the concentric electrode was 1.27 mm and ½ inch in length. We have used a concentric electrode for recording SSEP from the hind limb area of the somatosensory cortex.

Anaesthesia

Appropriate anaesthesia in the right dose is very much essential to get a reliable electrophysiological recording. We have chosen urethane as a deep anaesthetic agent. As it is deep and stable anaesthesia, it keeps the animal in anaesthesia for longer time and helps in getting an effective response. We injected urethane intraperitoneally at a dose of 1.5 g/kg body weight to anaesthetise the rats and waited 30 min to reach deep anaesthesia. After injecting anaesthesia, we ensured deep anaesthesia by pinching the footpad and tail of the animal to elicit a response. If there is no response after footpad and tail pinching, only then we proceed to the next level of the experiment.

Craniotomy and electrode placement

First, the rat was fixed in stereotaxic apparatus (model 1404, David Kopf, Tujunga, CA, USA), fur was removed and a single deep skin incision was made to expose the skull. The fascia was removed with scissors and any blood or tissue fluid was cleaned using a sponge. The skull was allowed to dry and bregma was identified. Bregma is a reference point junction of the coronal and sagittal sutures of the skull. As there is an overlap between rat cortical hind limb motor and sensory areas, we planned to make a window on the skull so that recording or stimulating electrodes can be moved easily into the brain for SSEP and MEP, respectively [Figure 1].

the We followed Paxinos atlas to identify the coordinates of hind limb representation on the cortex.[8,9] A window of 2 × 2 mm was prepared by removing overlying skull bone using a mechanical micro-drill. The utmost precaution was taken not to damage the underlying dura.

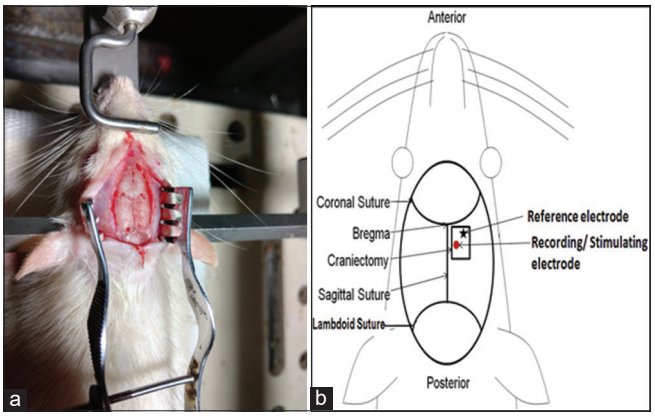

Figure 1: (a) Real image of a rat skull and position of Bregma and sutures. (b) Schematic diagram of placement of electrodes in somatosensory and motor cortex of hind limb.

Recording of MEP

After going through the literature for evoked potential recording for different animal species, we made a window from coordinates AP-1.0 mm, and ML-1.0 mm considering bregma as a reference point. The space within the window was filled with paraffin oil to keep the brain moist and to reduce noise from the surroundings. The needle electrode was fixed in the electrode holder and placed in the centre of the virtual grid made on the skull over the window of 0.5 mm in all axes. Then, the stimulating electrode for MEP was moved slowly from one end to the other covering all points on the grid. A needle electrode was used for stimulation in the Z-axis and the ground electrode was placed on the skull bone. Recording hook electrodes were used to enclose the sciatic nerve in the contralateral hind limb. Electrodes were secured firmly so that it does not make any injury to the nerve. Both the electrodes were covered in mineral oil to reduce the noise and insulate from the surrounding.

A constant current anodal stimulus was applied to the motor cortex for the generation of MEP. The parameters which were maintained to stimulate the motor cortex were as follows: Pulse duration = 300 μs, pulse rate 8 Hz, and stimulus intensity 0.1–11.2 mA. We used a band stop frequency of 1000 Hz of 400 Hz to visualise the response with the sampling rate of 100k/s and ten responses were averaged for each threshold point. The response to these stimuli was recorded from the sciatic nerve and gastrocnemius muscle as described above [Figure 2]. We observed maximum amplitude of MEP with minimum current at AP-2.5 mm, and ML-1.5 mm.

Recoding of SSEP

As stated earlier due to the overlap of hindlimb motor and sensory areas in Wistar rats, we recorded SSEP from various positions of the cortex in the open window as described

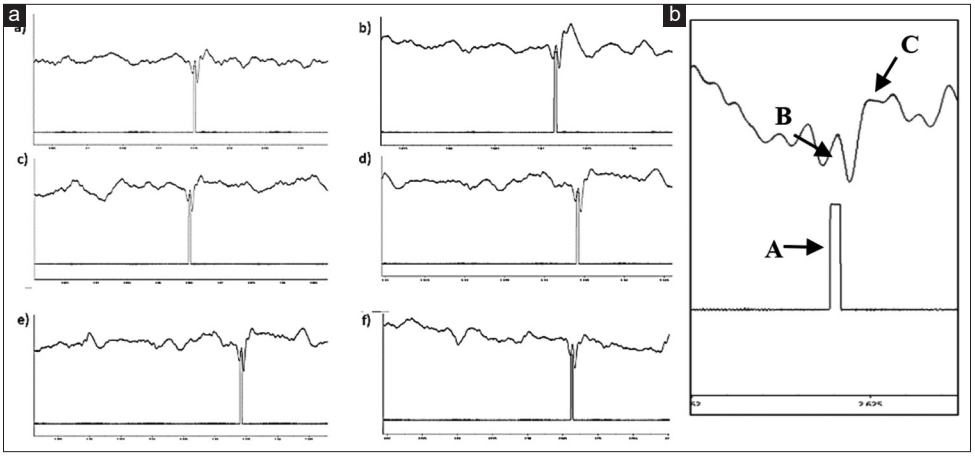

Figure 2: (a) Representative recordings of Motor evoked potential (MEP) in six rats. (b) magnified response of MEP. (A) Stimulus; (B) Stimulus artifact; (C) Response.

above. Stimulation was provided through the exposed sciatic nerve of the contralateral side with a pair of hook electrodes. A constant current stimulus was applied for the recording of SSEP from the sensorimotor cortical surface. A ground needle electrode was placed in the tail of the animal. Both the stimulating and recording electrodes were covered by mineral oil for insulation. The parameters which were maintained for stimulation were as follows: Pulse duration = 300 μs and stimulus intensity 0.1–11.2 mA. We used a band stop frequency of 1000 Hz of 400 Hz to visualise the response with the sampling rate of 100k/s. Ten responses were averaged for each threshold point. We observed maximum amplitude of SSEP with minimum current at the coordinates AP-2.5 mm and ML-2.8 mm [Figure 3].

Special highlights

1. We tried to ground the whole setup (stereotaxy, ADI stimulator, amplifier and recorder) as much as possible
2. All the experiments were performed in Faraday's cage
3. Utmost care was taken to prevent any damage to the underlying dura mater while making the window or placing electrodes.
4. It was taken care that no extra oil is near the electrodes. Skull was dried completely to ensure no blood or fluid in the vicinity
5. Minimum electrical gadgets were switched on in the electrophysiology laboratory and also the room was sound attenuated.

RESULTS

AD instrument amplifier and power lab are associated with software, LabChart v8.1.16. We have analysed data using peak to peak analysis feature of LabChart software.[10] We have found precise MEP and SSEP responses with minimum stimuli at AP-2.5 mm, ML-1.5 mm and AP-2.5 mm and ML-2.8 mm, respectively.

DISCUSSION

This *in vivo* technique is one of the oldest methods to understand the integrity of the neural system. The present study demonstrates a very reliable method of recording MEP and SSEP in anaesthetised Wistar male rats. These evoked responses provide an insight into the integrity and functional status of the brain and spinal cord.

We have observed a complete abolition of the motor as well as SSEP 5 weeks after severe contusion injury to the thoracic spinal cord.[10] We propose that this technique could be a reliable measure to assess the function of the sensory and motor system. Any intervention to improve motor or sensory function can also be assessed based on analysis of evoked potentials. Although recording of evoked potential in an anaesthetised animal pose limitation as different anaesthesia can alter cortical excitability or neuronal function to varying degrees,[11] eliciting MEP or SSEP in awake animals without significant restraint is also a herculean task. In electrophysiological studies, it is essential to achieve stable and deep anaesthesia for prolonged periods to obtain good reproducibility. Urethane is generally preferred in these studies which has the main advantage of providing stable anaesthesia for long durations as well as the induction of anaesthesia without altering neuronal plasticity to a significant extent.[12,13]

Our study will be beneficial for future researchers who are involved in the understanding of motor and sensory descending and ascending pathways. This method is dependent on some basic electrophysiological acquisition systems (a stimulation, an amplifier, and one recording system), which makes it more reachable for general neurophysiology laboratories.

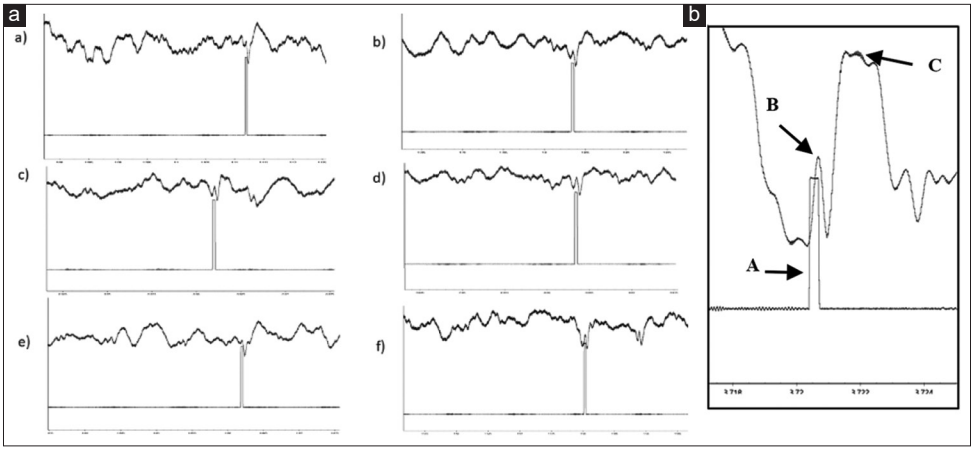

Figure 3: (a) Representative recordings of Somatosensory evoked potential (SSEP) in six rats. (b) magnified response of SSEP. (A) Stimulus; (B) Stimulus artifact; (C) Response.

CONCLUSION

The study describes in detail the protocol for recording motor and somatosensory evoked potentials in a Wistar male adult rat under deep anesthesia, which we presume would be extremely beneficial to all the neuroscientists involved in animal based research.

Acknowledgment

We would like to sincerely thank the Department of Science and technology (DST) for funding this work. Work has been conducted at All India Institute of Medical Sciences, New Delhi.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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