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RESEARCH ARTICLE

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Transcranial magnetic stimulation in the rat

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Abstract Transcranial magnetic stimulation (TMS) allows for quantification of motor system excitability. While routinely used in humans, application in other species is rare and little is known about the characteristics of animal TMS. The unique features of TMS, i.e., predominantly interneuronal stimulation at low intensity and non-invasiveness, are particularly useful in evaluating injury and recovery in animal models. This study was conducted to characterize the rodent motor evoked potential to TMS (MEP_{TMS}) and to develop a methodology for reproducible assessment of motor excitability in the rat. MEP_{TMS} were compared with responses evoked by electrical stimulation of cervical spinal cord (MEP_{CES}) and peripheral nerve. MEP were recorded by subcutaneous electrodes implanted bilaterally over the calf. Animals remained under propofol infusion and restrained in a stereotactic frame while TMS followed by CES measurements were obtained before and after 2 h of idle time. TMS was applied using a 5-cm-diameter figure-of-eight coil. MEP_{TMS} had onset latencies of 6.7±1.3 ms. Latencies decreased with higher stimulation intensity (r=–0.7, P<0.05). Two morphologies, $MEP_{TMS,\ 1}$ and $MEP_{TMS,\ 2}$, were distinguished by latency of the first negative peak (N1), overall shape, and amplitude. $MEP_{TMS,\ 2}$ were more frequent at higher stimulation intensity. While recruitment curves for $MEP_{TMS,\ 1}$ followed a sigmoid course, no supramaximal response was reached for $MEP_{TMS,\ 2}$. Mid-cervical spinal transection completely abolished any response to TMS. MEP_{CES} showed a significantly shorter latency (5.29±0.24, P<0.0001). Two types of MEP_{CES} resembling $MEP_{TMS,\ 1\ and\ 2}$ were observed. Neither MEP_{TMS} nor MEP_{CES} changed on repeat assessment after 2 h. This study demonstrates the feasibility and reproducibility of TMS in the rat. Sigmoid recruitment curves for $MEP_{TMS,\ 1}$ suggest input-output properties similar to those of the human corticospinal system. Latency differences between CES and TMS point to a supraspinal origin of the MEP_{TMS}. The two morphologies likely reflect different cortical or subcortical origins of MEP_{TMS}.

Keywords Transcranial magnetic stimulation · Corticospinal excitability · Rat

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Introduction

Transcranial magnetic stimulation (TMS) is routinely used in humans for a variety of clinical and scientific applications. TMS applied to the motor cortex allows for painless stimulation of the corticospinal tract eliciting motor evoked potentials (MEP) in peripheral muscle. In recent years, this technique has provided important insight into basic motor physiology (Cracco et al. 1999). Corticospinal excitability can be quantified using TMS. Different interventions such as motor training (Classen et al. 1998) or deafferentiation (Ziemann et al. 1998) have been shown to persistently alter excitability. Modulation of excitability is therefore regarded as an indicator of early neuroplastic change (Cohen et al. 1998; Pascual-Leone et al. 1999).

In contrast to its widespread use in humans, TMS is rarely applied to animals. Spinal cord injury (Magnuson et al. 1999) and anesthesia (Ebert and Ziemann 1999) have been assessed using TMS in the rat. Animal studies have elucidated TMS mechanisms (Wang et al. 1996) and demonstrated its safety (Russell et al. 1994; Post et al. 1999).

Studies on motor physiology commonly use electrical stimulation, which is easier to perform without specialized equipment. However, the unique features of TMS, i.e., predominantly interneuronal rather than pyramidal stimulation at low intensity (Cracco and Cracco 1999), define it as a distinct methodology. Its non-invasiveness not only allows for evaluation of injury and recovery in animal models, but also enables repeated assessments of excitability as a measure of early neuroplastic changes in animals.

This study was conducted to characterize the rodent motor potential evoked by TMS (MEP_{TMS}). A methodology is presented that allows for reliable and repetitive application of TMS in the rat. Differences and similarities to TMS in humans are discussed.

Materials and methods

TMS was applied to 21 adult male Wistar rats (300–400 g body weight) while the animals were anesthetized and fixated in a stereotactic frame. The electromyogram (EMG) was recorded bilaterally from the calf muscles (gastrocnemius). MEP_{TMS} were compared with motor potentials evoked by electrical stimulation of the cervical spinal cord (MEP_{CES}). The reproducibility of MEP_{TMS} and MEP_{CES} was evaluated by repeating the stimulation after 2 h while the animal remained sedated and in a fixed position. All animal procedures were approved by the institutional Animal Care Committee of the Johns Hopkins University and were in accordance with NIH guidelines.

Animal preparation

For surgical placement of electrodes and venous access, animals were anesthetized with halothane (induction 2.5%, maintenance 2% dissolved in 30%/70% oxygen/nitrogen applied via nose cone). Anesthesia depth was adjusted for absence of abdominal contractions to tail pinch. Body temperature was monitored rectally and maintained by a water-circulation heating pad. A venous catheter (24 G) was inserted into the lateral tail vein. Two EEG needle electrodes (Grass, Astro-Med Inc., West Warwick, RI) were placed symmetrically into the scalp of the forehead (parallel to the surface of the bone, parallel and 3–4 mm to both sides of the sagittal suture, needle tip 2–3 mm anterior to the coronal suture). EKG needle electrodes were implanted into the right forepaw and the left hindpaw. EMG electrodes consisted of folded chlorinated silver wire forming flat plates 1×0.5 cm^2 in area (Fig. 1a). EMG electrodes were implanted bilaterally into a subcutaneous pocket over the calf muscles. Straight wires placed below the ankle served as EMG reference electrodes. Spinal stimulation electrodes were made from straight tungsten wire and were placed on both sides of the cervical spine caudal to the mastoid bone. After infiltrating the external auditory meatus with bupivacaine, animals were transferred into a stereotactic frame and continuous recording of EEG, bilateral EMG, and EKG was started.

Subsequently, animals were loaded with intravenous propofol (10 mg/kg over 15 min). Five minutes after starting application of the loading dose, halothane was turned off. Propofol sedation was maintained using infusion rates of 400–700 µg/kg/min. The infusion rate was titrated to suppress spontaneous activity in hindlimb EMG recordings. Oxygen was supplemented at 0.8 l/min via nose cone.

TMS measurements were started 1–2 h after termination of halothane to ensure complete washout (Luschei and Mehaffey 1967). The washout process was monitored by online EEG spectral power analysis. Relative power in different frequency bands (1–3 Hz, 4–7 Hz, 8–12 Hz, 13–17 Hz, 18–22 Hz, 23–27 Hz) was plotted over time. At the end of the experiment, animals were put to death by an overdose of pentobarbital (1 mg, i.v.).

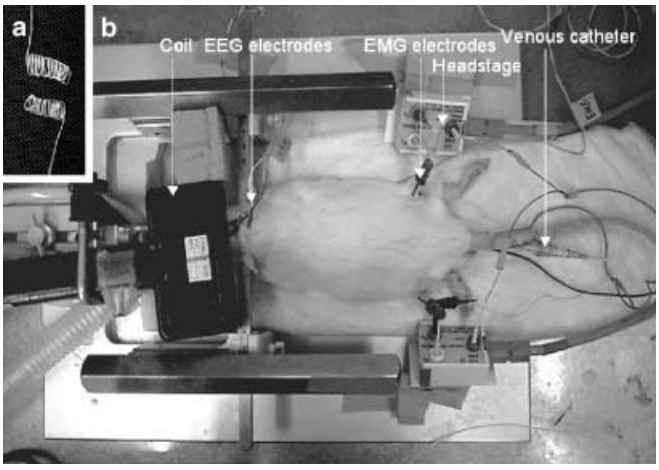

Fig. 1a, b Experimental setup for measuring motor potentials in bilateral calf muscles in response to transcranial magnetic stimulation (TMS). **a** Electrodes implanted into a subcutaneous pocket over the calf muscle bulk consisted of chlorinated silver wire that was folded to form flat plates. **b** The TMS coil (figure-of-eight coil encased in a black box) was placed asymmetrically over the rat's forehead 0.5 cm lateral to the bregma. During the entire experiment, bilateral calf EMG, EEG, and EKG were recorded. Propofol was administered via a venous catheter placed into a lateral tail vein

Transcranial magnetic stimulation

TMS was administered using a Cadwell stimulator (MES 10, Cadwell Laboratories, Kennewick, WA) delivering biphasic stimuli via a figure-of-eight coil (5 cm coil diameter). The magnetic coil was mounted to the stereotactic frame (Fig. 1b). The center of the coil ("hotspot") was positioned 0.5 cm lateral to the bregma. The coil was moved craniocaudally (±0.5 cm relative to the bregma) to optimize MEP_{TMS} responses. The coil was not angulated, but placed flat onto the calvarial bone.

Each TMS assessment included determination of motor threshold (MT) followed by recording of 20 stimuli for each of two stimulation intensities (130% and 150% relative to MT). MT was determined by increasing magnetic stimulation intensity in steps of 4 percentage points of absolute stimulator output starting with intensities that did not produce MEP_{TMS} responses. Ten MEP_{TMS} were recorded for each intensity level (7 s interstimulus interval). MEP_{TMS} with an amplitude ≥15 µV were considered suprathreshold. MEP with amplitudes of 15 µV were visually identifiable above noise level and were typically found in the initial horizontal part of the recruitment curve. The intensity was increased until 10 of 10 suprathreshold MEP_{TMS} were observed. MT was subsequently defined as the intensity (rounded to the nearest 1% of maximum stimulator output) producing 50% suprathreshold responses. After MT determination, 20 MEP_{TMS} were recorded for each of two intensities (130% and 150% relative to MT) with an interstimulus interval of 30 s. This interval was chosen to avoid overheating of the coil at high stimulation intensities. A complete set of TMS measurements lasted 33±8 min. Initial MT in two rats was too high to allow stimulation at 150% relative to MT; these rats were excluded from further analysis, reducing the study group to ten animals.

Cervical electrical stimulation

Electrical stimulation of the cervical spinal cord (CES) was performed in five animals of the study group. Pulses of 0.9 ms length were applied with intensities ranging between 2 and 20 V (constant voltage stimulation, Grass, Astro-Med Inc., West War-