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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 $MEP_{TMS,\,1\text{ and }2}$ 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

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).

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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.).

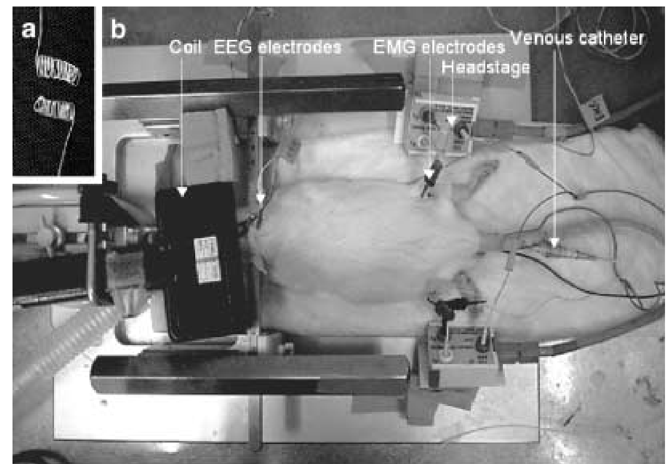

Fig. 1a, b Experimental setup for measuring motor potentials in bilateral calf muscles in response to transcranial magnetic stimulation (TMS). **a** Electrodes implanted into a subcutaneous pocket over the calf muscle bulk consisted of chlorinated silver wire that was folded to form flat plates. **b** 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-

wick, RI). Ten MEP_{CES} were recorded at three or more levels of intensity. Starting at 2 V, the intensity was increased in steps of 1 V.

Experiments

The following experiments were conducted:

Six experiments aimed to identify the anesthetic regimen most suitable for repeated application of TMS. Tested anesthetics included halothane (one animal, 1.5–2.0% in 30% O_2/70% NO_2 continued after surgery via nose cone), pentobarbital (one animal, 1 mg/kg body weight intraperitoneal bolus injection, supplementary doses 0.5–1 mg/kg), ketamine (two animals, 10 mg/kg body weight/h continuous intravenous infusion), and propofol (two animals, according to the above described protocol). After identifying propofol as the anesthetic of choice, respiratory sufficiency was tested in four propofol-anesthetized animals. The ventral tail artery was cannulated with PE50 tubing and blood samples were obtained every hour. These animals received TMS at 15 stimulation intensities (starting at 36% below MT, intensity was increased in steps of 7%) to allow recording of recruitment curves. Also, TMS was repeated after 2 h of idle time and these animals were included in the study group. Eight additional animals undergoing two TMS trials were added to the study group (two excluded for high MT resulting in total n=10), five of which received CES.

The procedure to determine the motor threshold was tested in one animal. MT was assessed 4 times over a period of 4 h. In one additional animal, responses to stimulation of different modalities, i.e., TMS over bregma, TMS 2 cm caudal to bregma, electrical stimulation of cervical spine and peripheral nerve, were recorded.

In one additional animal, transection of the spinal cord was performed at the C7 level to assess whether motor evoked responses were specific to calf muscle and not volume-conducted artifacts from the site of magnetic stimulation. This rat was endotracheally intubated using a 14-G plastic tube and maintained on a rodent ventilator under constant-volume ventilation (45 breaths/min, volume: 1 ml/100 g body weight). As described above, halothane was exchanged for propofol before baseline TMS measurements were obtained. Before transection surgery, halothane was restarted and later weaned for postsurgical TMS assessment.

Recording and data analysis

EMG (amplification: 2×10^4; high-pass: 1 Hz; low-pass: 5 kHz; sampling rate: 10 kHz; Grass amplifiers and ×10 headstage; Astro-Med Inc., West Warwick, RI), EEG, and EKG were continuously recorded throughout the experiment. MEP peak-to-peak amplitudes, onset latency (point of initial deflection from baseline), and latency of first negative peak (N1) were measured using Spike2 software (CED Ltd., Cambridge, UK). Statistical analyses were performed using SPSS software (SPSS Inc, Chicago, IL). For each animal, each timepoint, and each intensity of stimulation, averages were computed for values derived from single MEP sweeps (e.g., mean latency, mean peak-to-peak amplitude). Statistical tests were applied to average values. Paired t-tests were computed for side-to-side comparisons, whereas unpaired t-tests were used to detect differences in amplitude and latency between MEP_{TMS} of different types ($MEP_{TMS,\ 1}$ and $MEP_{TMS,\ 2}$) and between MEP_{TMS} and MEP_{CES}. A repeated-measures ANOVA was used to test for differences before and after 2 h of idle time (one-way, MEP amplitude as dependent factor). Regression analysis was performed to estimate the relationships between stimulation intensity and amplitude/latency/frequency of $MEP_{TMS,\ 2}$. Average values given in the text are expressed ± standard deviations. Probability values present two-tailed estimates.

Results

Anesthesia and sedation

Recording of motor evoked potentials from relaxed muscle required sedation of the animal. Low-dose halothane, pentobarbital, ketamine, and propofol were tested for this purpose. Halothane and pentobarbital abolished the MEP_{TMS} in anesthetizing concentrations. Under ketamine, MEP_{TMS} were initially present, but significantly dampened or eliminated after 3 h of continuous administration. Propofol was identified as the drug of choice because it maintained stable MEP_{TMS} responses over a period of 4 h. The MEP_{TMS} was hardly affected by propofol infusion rate, if maintenance infusion was kept below 700 µg/kg/min. Above this rate, MT increased. However, while titrating propofol infusion rate to suppress spontaneous EMG activity, none of the rats required rates >700 µg/kg/min to remain sufficiently sedated and relaxed.

Since propofol has poor analgesic properties, preparative surgery had to be performed with the animal under halothane. The animal was then shifted from halothane to propofol to allow MEP recording. This shift was monitored using online plotting of EEG band power. Halothane washout was accompanied by a reduction of low frequency power, while high frequencies became more prominent (Fig. 2). TMS measurements were commenced after EEG power reached constant levels. This was commonly achieved 30–45 min after halothane was turned off. TMS measurements were started 1 h after halothane cessation to ensure constancy of power bands.

This anesthetic protocol did not affect the animal's oxygenation. Repeated arterial blood gas samples demonstrated constant values in the normal range (Table 1). There was no evidence of acidosis, hypoxemia, or hypercapnia during a 4-h period.

Motor threshold

The MT was measured in ten rats at baseline and after 2 h. MT showed high interindividual variability (baseline mean MT±SD: 42.8±19.1 points of absolute stimulator output; 2-h MT: 52.9±19.9). Within animals, MT was relatively stable (mean absolute change 16±10%); a paired comparison between baseline and 2-h MT did not yield significant differences.

One pilot experiment was conducted to further investigate the applicability of the MT concept in the rat. Four

Table 1 Arterial blood gas values during continuous propofol administration in four animals

	Baseline	1 h	3 h	4 h
pH	7.47±0.05	7.45±0.03	7.45±0.01	7.45±0.02
pCO_2 (mmHg)	22.7±1.42	25.6±4.3	24.2±3.7	32.5±6.5
pO_2 (mmHg)	90.3±23.0	92.0±16.1	91.9±10.5	86.2±11.2
O_2 saturation (%)	96.9±1.2	97.3±0.8	97.4±0.6	96.9±0.7

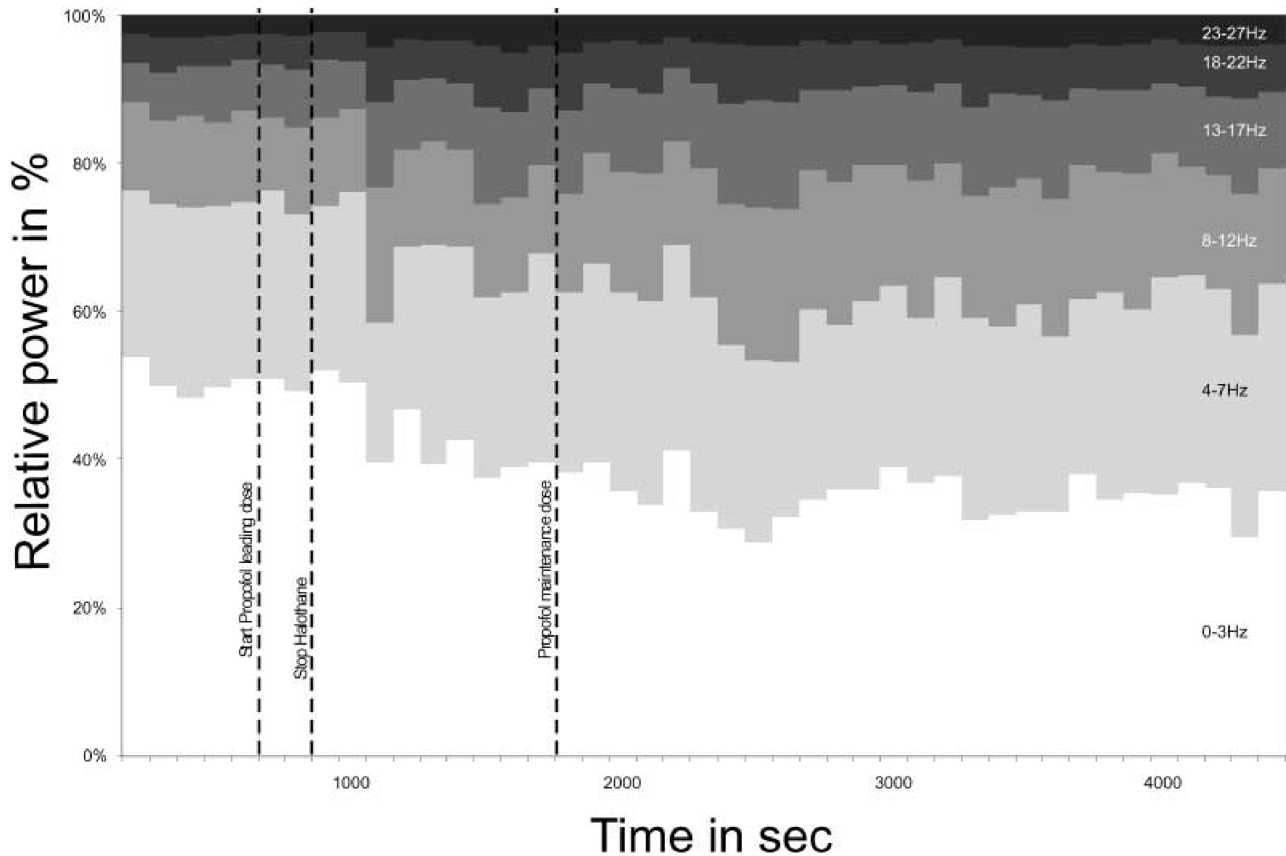

Fig. 2 Monitoring of anesthesia during the experiment. Relative power in different EEG frequency bands is plotted against time (stacked layout: for each timepoint; relative power values add up to 100%). Halothane was used for surgical preparation and was replaced by propofol before TMS measurements were started. The transition from halothane to propofol was monitored by online EEG power analysis, an example of which is shown here. As soon as halothane was stopped, low frequency power decreased while higher frequencies became more prominent. Frequencies remained constant during continuous propofol maintenance infusion

MT assessments were performed at baseline, 120 min, 180 min, and 240 min after initiation of propofol. For each assessment, the frequency of suprathreshold MEP_{TMS} was plotted against stimulation intensity. At all timepoints sigmoid curves were observed (Fig. 3).

Features of motor potentials to transcranial magnetic stimulation (MEP_{TMS})

Based on amplitude and morphology, two distinct types of MEP_{TMS} were identified (Fig. 4). The first ($MEP_{TMS,\ 1}$) consisted of an initial negative deflection (N1) followed by one or two positive peaks (P1, latency: 9.65±0.2 ms; and P2, latency: 11.86±0.2 ms) of lower amplitude (Tables 2, 3). $MEP_{TMS,\ 2}$ had larger amplitudes, had higher N1 latencies, and lacked the P2 peak (Fig. 4c). The MEP in some trials seemed to begin with an $MEP_{TMS,\ 1}$ waveshape which was suddenly interrupted by $MEP_{TMS,\ 2}$, i.e., the N1 peak of $MEP_{TMS,\ 1}$ was not completed when the $MEP_{TMS,\ 2}$ N1 occurred (Fig. 4a, middle trace). This phenomenon was observed when the $MEP_{TMS,\ 2}$ amplitude was relatively small (as compared to other $MEP_{TMS,\ 2}$). For larger $MEP_{TMS,\ 2}$, the N1 began earlier, making it difficult to appreciate an $MEP_{TMS,\ 1}$ N1 peak inside the $MEP_{TMS,\ 2}$ N1. There was a smooth transition from "inter-

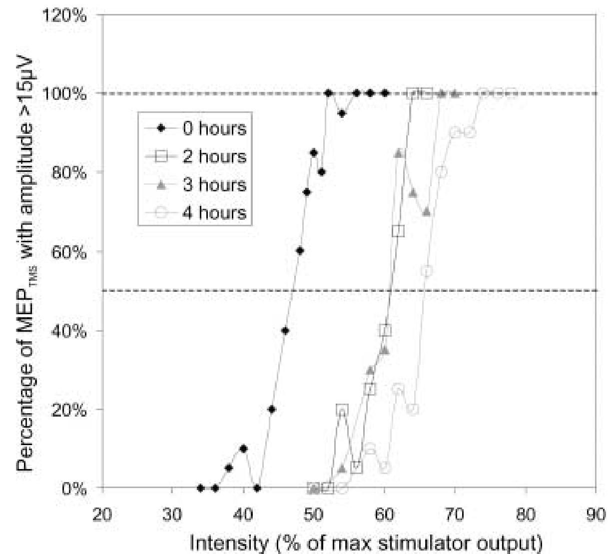

Fig. 3 One experiment was conducted in a single animal to test the applicability of the MT concept in the rat. MT was serially measured over a period of 4 h. For each assessment, the frequency of suprathreshold MEP_{TMS} (as defined by amplitude ≥15 μV) was found to have a sigmoid relationship to stimulation intensity. The MT can therefore be defined – as in humans – as the intensity at which suprathreshold MEP_{TMS} are evoked with 50% probability. Between zero and 2 h, during which steady state of propofol anesthesia is reached, a shift in MT is noted. Thereafter, MT remains constant

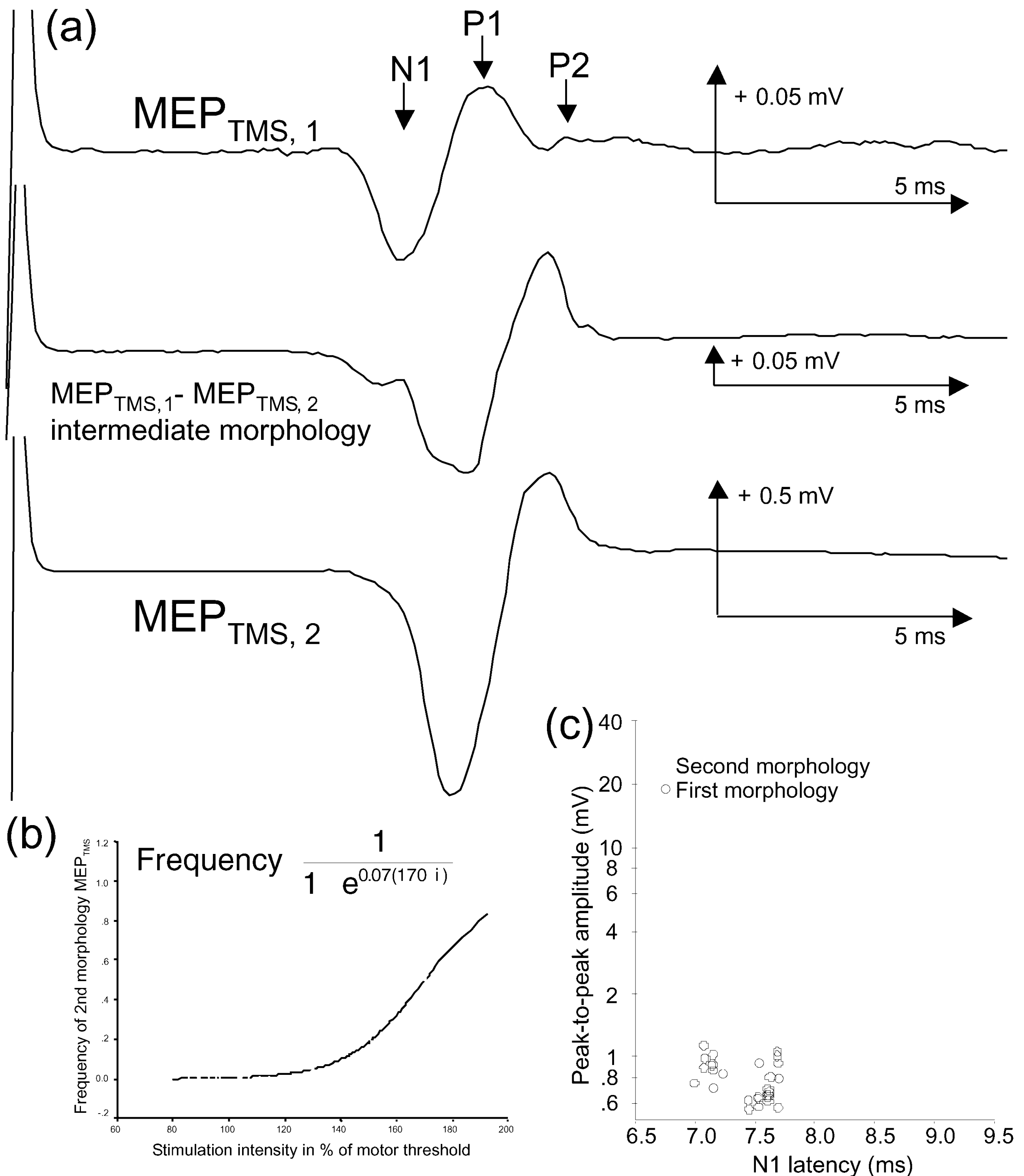

Fig. 4a–c Features of the motor potential evoked by transcranial magnetic stimulation (MEP_{TMS}). **a** Each trace represents an average of ten MEP_{TMS} sweeps. The typical MEP_{TMS} ($MEP_{TMS,\,1}$, *top trace*) consisted of an initial negative peak (*N1*) followed by two positive deflections (*P1* and *P2*). A second MEP_{TMS} morphology ($MEP_{TMS,\,2}$) was identified and distinguished by the absence of P2, a higher amplitude (note different scales) and longer N1 latency (*bottom trace*). In some trials, both morphologies were identifiable (*middle trace*). **b** $MEP_{TMS,\,2}$ were more frequent at higher stimulation intensities. This relationship followed a sigmoid course (Boltzman's equation). **c** Based on the parameters "amplitude" and "N1 latency," both morphologies were clearly differentiated (shown here for one animal at a stimulation intensity of 50% above MT)

mediate" forms to "typical" $MEP_{TMS,\,2}$ that did not allow a clear separation of the two. Intermediates were hence classified as $MEP_{TMS,\,2}$. At 130% relative to MT, $MEP_{TMS,\,2}$ occurred with an average frequency of 3.2±4.8% in contralateral and 12.7±28.2% in ipsilateral recordings. The $MEP_{TMS,\,2}$ frequency significantly increased at 150% relative to MT (contra: 19.7±28.9%, ipsi: 23.2±33.9%, $P<0.05$). Side-to-side differences in frequency were nonsignificant. In 14% of trials $MEP_{TMS,\,2}$ were present bilaterally. The frequency of $MEP_{TMS,\,2}$ was highly variable among animals (0–100%), but significantly in-

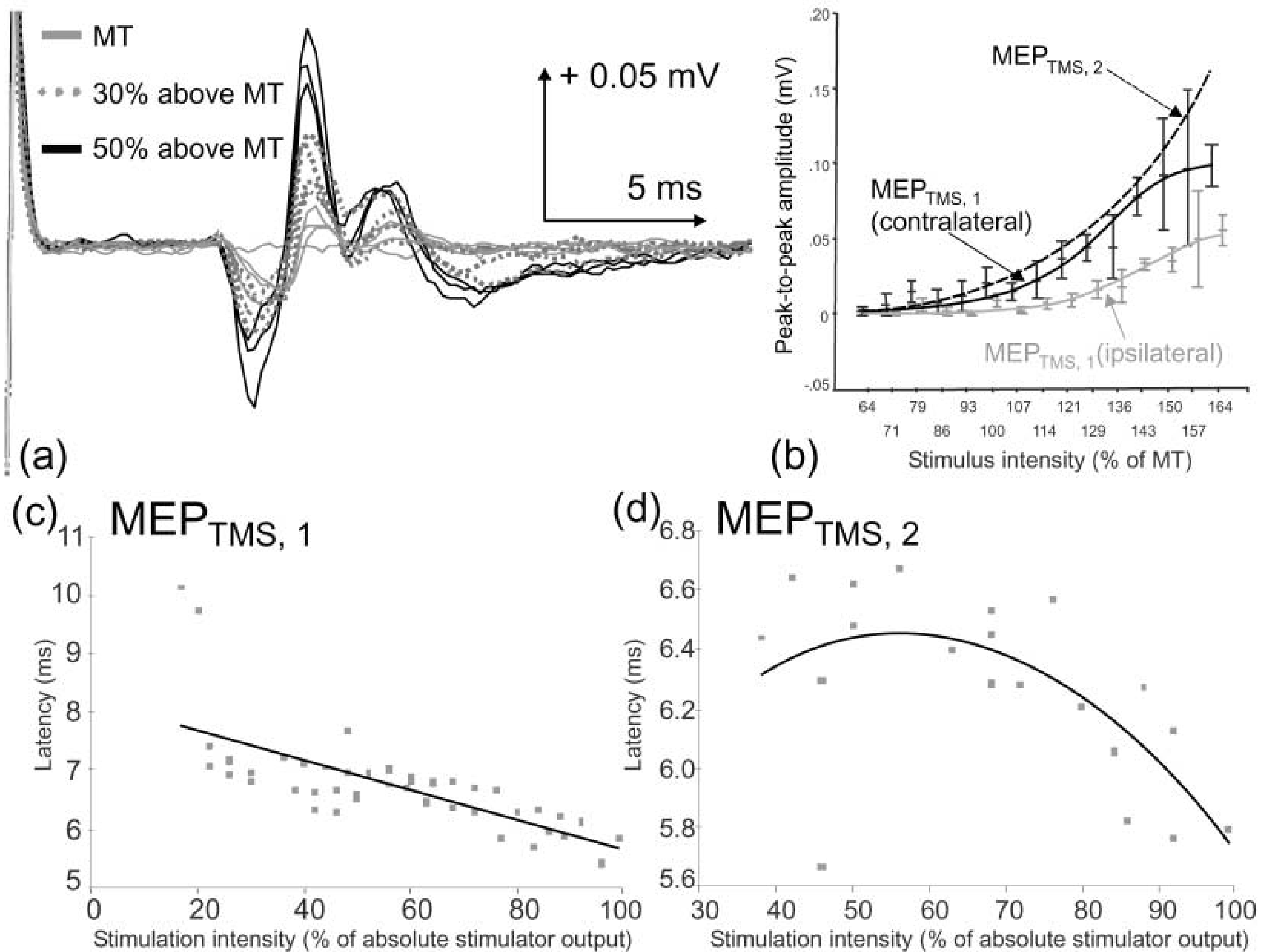

Fig. 5a–d Relationship between MEP$_{TMS}$ and stimulus intensity (data from one exemplary animal). **a** Sweeps of MEP$_{TMS, 1}$ acquired with three different stimulation intensities are superimposed. The amplitude of the MEP$_{TMS, 1}$ increases with stimulation intensity. **b** The relationship between MEP$_{TMS, 1}$ amplitude and stimulus intensity (recruitment curve) follows a sigmoid course. For MEP$_{TMS, 2}$, the recruitment curve does not plateau, indicating that no supramaximal stimulation is achieved. **c** A linear decrease in MEP$_{TMS, 1}$ latency was found with a higher stimulation intensity (r=–0.70, P<0.0001). **d** For MEP$_{TMS, 2}$, that relationship was best approximated by a second order function (R^2=0.48, P<0.01 as compared to linear R^2=0.23, P<0.05)

creased with stimulation intensity in a sigmoid fashion (Boltzman's equation, R^2=0.44), demonstrating that most MEP$_{TMS, 2}$ occur at stimulation intensities ≥140% relative to MT (Fig. 4b).

Amplitudes and latencies for either MEP$_{TMS, 1}$ or MEP$_{TMS, 2}$ did not differ from side to side (Tables 2, 3). After 2 h of idle time, MEP$_{TMS}$ amplitude was slightly, but non-significantly, reduced for MEP$_{TMS, 1}$ (–24% at a stimulation intensity of 130% MT, –9% at 150% MT, NS) and increased for MEP$_{TMS, 2}$ (+58% at 130% MT, +3% at 150% MT, NS). There were no differences in onset or N1 latencies.

MEP$_{TMS, 1}$ amplitude increased with stimulation intensity following a sigmoid course (recruitment curve, R^2=0.36, Fig. 5a, b). In contrast, MEP$_{TMS, 2}$ amplitude did not plateau even at maximum stimulator output (Fig. 5b). Latencies of both MEP$_{TMS}$ decreased with absolute stimulation intensity (Fig. 5c). Whereas the regression between onset latency and intensity was linear for MEP$_{TMS, 1}$ (Pearson's r=–0.7, P<0.0001, R^2=0.49), a second-order relationship was found for MEP$_{TMS, 2}$ (R^2=0.48, P<0.01 as compared to a linear relationship R^2=0.23, P<0.05, Fig. 5d).

In one animal, TMS was applied at a midline position 2 cm caudal to the bregma. The resulting MEP$_{TMS}$ (only MEP$_{TMS, 1}$ were obtained in this animal for any coil position) had significantly shorter latencies than MEP$_{TMS}$ obtained by stimulation over the bregma (P<0.01, Fig. 6a, b).

Spinal transection led to complete extinction of MEP$_{TMS}$ of either morphology. While moving the coil caudally, no MEP$_{TMS}$ were evoked until the distal stump of the spinal cord was reached.

Features of motor potentials to electrical stimulation (MEP$_{CES}$ and M response)

Two types, similar to MEP$_{TMS, 1}$ and MEP$_{TMS, 2}$, were observed for MEP$_{CES}$. The MEP$_{CES, 1}$ consisted of N1 and P1; P2, however, was dampened as compared to MEP$_{TMS, 1}$ (Fig. 6). Latencies of MEP$_{CES, 1}$ were significantly shorter

Table 2 MEP characteristics at baseline: MEP_{TMS} (average of ten animals)

TMS intensity (% relative to MT)		$MEP_{TMS, 1}$			$MEP_{TMS, 2}$		
		Onset latency (ms)	N1 latency (ms)	Amplitude (μV)	Onset latency (ms)	N1 latency (ms)	Amplitude (μV)
130%	Contra	6.74±1.26	8.39±1.26	61±68	6.46±0.49	9.45±0.95	507±626
	Ipsi	6.61±1.01	8.25±1.08	61±129	6.24±0.36	9.74±0.80	546±426
150%	Contra	6.62±1.29	8.32±1.27	82±59	6.36±0.39	9.45±0.82	398±356
	Ipsi	6.40±0.83	8.16±0.93	70±74	6.22±0.48	9.50±0.88	680±754

Table 3 MEP characteristics at baseline: MEP_{CES} (average of five animals)

CES intensity (V)		$MEP_{CES, 1}$			$MEP_{CES, 2}$		
		Onset latency (ms)	N1 latency (ms)	Amplitude (μV)	Onset latency (ms)	N1 latency (ms)	Amplitude (μV)
4	Contra	5.29±0.24	6.57±0.20	54±68	4.95±0.37	7.56±0.37	646±116
	Ipsi	5.41±0.23	6.67±0.33	37±22	4.72±0.51	7.76±0.62	504±220

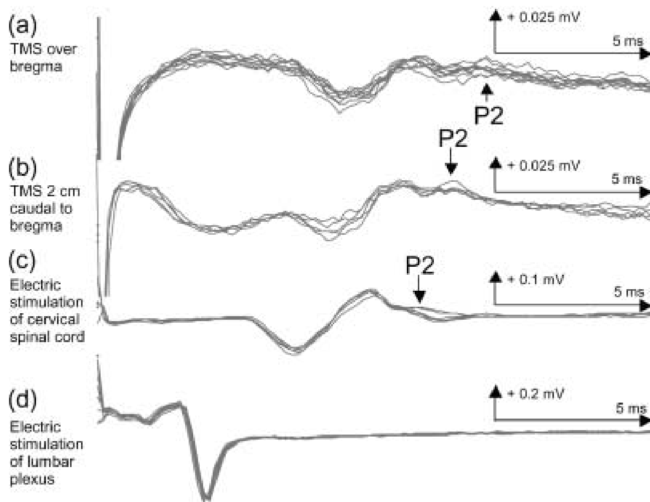

Fig. 6a–d Comparison of MEP in response to transcranial magnetic (TMS) and electric stimulation (CES) to different target structures. For each modality, ten traces are superimposed. All MEP_{TMS} shown are examples of $MEP_{TMS, 1}$ (see Fig. 4). **a** MEP_{TMS} evoked by stimulation over the bregma have slightly longer latencies than **b** MEP_{TMS} resulting from stimulation 2 cm caudal to the bregma using the same stimulation intensity. **c** Electric stimulation of cervical spinal cord results in MEP with similar signal lengths as MEP_{TMS}, but shorter latency. The P2 peak of MEP_{TMS} is present in MEP_{CES} but is less prominent. **d** The peripheral muscle response (M-response) to electric stimulation of the lumbar plexus has a much shorter latency and signal length as compared to MEP_{CES}

than latencies of $MEP_{TMS, 1}$ ($P<0.0001$, Fig. 6, Tables 2, 3). Latency distributions of $MEP_{CES, 1}$ and $MEP_{TMS, 1}$ did not overlap. The amplitude of $MEP_{CES, 1}$ increased with stimulation intensity (Pearson's $r=0.66$, $P<0.0001$), commonly reaching a plateau at 12–15 V. $MEP_{CES, 2}$ – as differentiated by longer N1 latency and higher amplitude ($P<0.0001$) – highly resembled the shape of $MEP_{TMS, 2}$, but had significantly shorter latencies ($P<0.0001$). $MEP_{CES, 2}$ occurred in 11±10% and 18±19% of all contralateral and ipsilateral trials, respectively. The frequency of $MEP_{CES, 2}$ increased with stimulation intensity following a sigmoid course ($R^2_{sigmoid}=0.69$, $P<0.01$ as compared to $R^2_{linear}=0.16$, NS). No changes in MEP_{CES} amplitude or latencies were found after 2 h of idle time.

Lumbar plexus stimulation evoked M-responses in the ipsilateral hindlimb (Fig. 6d). Their latency was markedly shorter (onset: 2.70±0.05 ms, N1: 3.62±0.05 ms) as compared to MEP_{CES} or MEP_{TMS}.

Discussion

This study describes the features of the motor evoked potential elicited by TMS in rat and presents a methodology for reproducible assessment of rodent corticospinal excitability.

Animal sedation

TMS in small animals requires muscle relaxation. Although MEP_{TMS} were successfully recorded in awake animals in the past (Linden et al. 1999), muscle contraction may influence MEP_{TMS} responses in rats as it does in humans (Mazzocchio et al. 1994). Contraction may confound the comparison between two TMS measurements. Therefore, we chose to sedate the animal. Most sedatives, however, reduce neuronal excitability and synaptic transmission, which dampens or eliminates muscle responses to TMS. This is a likely explanation for the inability to generate MEP_{TMS} under halothane and pentobarbital in this and other studies (Chiba et al. 1998).

Since one TMS assessment lasts about 30 min, sedation should be stable over time. Stability is also required for repeated TMS assessment. Ketamine was found to suppress the MEP_{TMS} after long periods of administra-

tion, which may be explained by accumulation of metabolites. Nevertheless, ketamine is a useful agent for a single TMS trial. Propofol did not suppress MEP_{TMS} even after prolonged administration. Though propofol has known inhibitory effects on neuromuscular transmission (Abdel-Zaher and Askar 1997; Keller et al. 1992), it was previously reported to minimally affect the MEP in small doses (Fishback et al. 1995). Rapid pharmacokinetics make it easy to adjust and wean. EEG monitoring and frequency analysis was found to be well suited for assessing the stability of sedation and the transition from halothane to propofol.

Features of the MEP_{TMS}

Especially in small animals like the rat, volume-conducted artifacts may obscure responses of local muscles. Spinal transection led to complete extinction of stimulus locked potentials in our setup. Therefore, volume-conducted transmission from the site of stimulation (TMS directly stimulating cervical muscles which then conduct the stimulus caudally to the recording site) seems unlikely. However, MEP_{TMS} may still be contaminated by potentials of large muscles other than the recorded one, which are excited via the neural route. Two aspects speak against this source of artifact: (1) TMS led to little visible muscle twitch across the body of the rat. It seems unlikely that distant muscles would dominate the calf response generated immediately under the recording electrode. (2) The initial negative deflection of the MEP_{TMS} supports location specificity of EMG recordings. Given the distal placement of the reference electrode, evoked potentials originating in the target muscle below the active electrode are expected to begin with a negative deflection (Kimura 1989). This suggests that at least the initial negative deflection (N1) reflects the response of local muscles while later peaks (especially high amplitude deflections of $MEP_{TMS,\ 2}$) may be artifacts.

We found two different types of MEP_{TMS} with characteristic morphologies, which to our knowledge were not reported previously. Other studies found bilateral MEP_{TMS} with similar latency and amplitude resembling the $MEP_{TMS,\ 2}$ observed in this study (Linden et al. 1999; Kamida et al. 1998; Fishback et al. 1995). The authors do not mention $MEP_{TMS,\ 1}$ responses with amplitudes in the microvolt range, which might have been missed due to low amplification of the EMG signal (×5000 as compared to ×20,000 in this study). The relationship between stimulus intensity and MEP latency observed in this study was also reported for transcranial electric stimulation (Dull et al. 1990; Zappulla et al. 1988).

Origin of MEP_{TMS}

Given the size of the coil in relation to the rat brain, the stimulus not only excited the hemisphere over which the coil was placed, but likely also contralateral regions. Brainstem and cerebellum may have been excited by stimulus spread.

Our results suggest that the excitable structure responsible for the generation of MEP_{TMS} is rostral to the cervical spinal cord: MEP_{TMS} had significantly longer latencies than MEP_{CES}. The fact that two different types of MEP_{TMS} were observed with magnetic as well as with electric stimulation suggests the involvement of two generators. These generators may be independent structures routing the stimulus through two different pathways. Alternatively, two morphologies may reflect the same pathway and differ in the amount of recruitment of fibers beyond a certain threshold of stimulation. However, the first explanation seems more likely, since MEP_{TMS} types are clearly separated by their amplitude and latency distributions (Fig. 4c). Their superposition in some trials (Fig. 4a, middle trace) further supports the notion of two independent origins.

Previously, MEPs induced by TMS in rat have been attributed to the stimulation of structures other than motor cortex (Kamida et al. 1998), because transecting the dorsal corticomotoneuronal tract, which represents the main corticomotoneuronal tract in the rat comparable to the pyramidal tract in the human (Brown 1971), did not abolish magnetic MEPs. In contrast, bipolar electrical transcranial stimulation over the motor cortex elicits longer-latency MEPs that disappear after transection of the dorsal corticomotoneuronal tract (Ryder et al. 1991). These studies suggested that the dorsal corticomotoneuronal tract in the rat is a slow pathway that is not substantially excited by TMS (Zappulla et al. 1991). However, motor cortex microstimulation – which can be considered as most focal to motor cortex – also exhibits short latency EMG responses (Liang et al. 1993), suggesting mono- or oligosynaptic pathways. MEP_{TMS} observed in this study had similar short latencies. The lesser penetration depth of the magnetic stimulus at low intensities makes cortex or cortical descending fibers a likely origin of $MEP_{TMS,\ 1}$. Higher intensities can excite deeper structures which may account for $MEP_{TMS,\ 2}$; deeper structures might also have been reached by CES to produce $MEP_{CES,\ 2}$. Overall, our data do not allow a definitive identification of the cerebral structure responsible for the generation of MEP_{TMS}. As compared to results obtained by others (Liang et al. 1993), neither cortex, brainstem, nor cerebellum can be excluded (Zappulla et al. 1988; Hurlbert et al. 1992). However, the data suggest that TMS excites fast pathways, which may reflect rodent motor function more accurately than the dorsal corticomotoneuronal tract.

The bilaterality of MEP_{TMS} responses observed in this study is most likely explained by excitation of the contralateral hemisphere. Stimulation of descending pathways distal to their decussation or partial decussation of descending tracts (Goodman et al. 1966) may also contribute to this finding. Hemispherectomy experiments are required to resolve this issue.

Comparison with human TMS

Recruitment curves obtained in rats demonstrated a sigmoid relationship between stimulus intensity and MEP_{TMS} amplitude. This relationship can also be observed in humans, indicating comparable input-output properties of the corticospinal pathway (Devanne et al. 1997).

In humans, TMS is assumed to preferentially excite motor cortex interneurons rather than pyramidal cells (Cracco and Cracco 1999; Terao et al. 2000). The electric field induced by magnetic pulses spreads parallel to the hemispheric surface, thereby stimulating cells with preferential horizontal extension (Cracco et al. 1999). The axis of pyramidal cells, however, is perpendicular to the cortical surface. Interneuronal stimulation is thought to produce (indirect) I-waves with longer latencies than (direct) D-waves, which are the results of pyramidal stimulation (Cracco et al. 1999). The human MEP_{TMS} is a combination of D- and I-waves depending on the individual, the cortical region being stimulated, and the stimulus intensity. In rat, different responses with latencies of 1.2 ms and 11–14 ms were observed in spinal recordings after electrical stimulation of cortex. Late responses were interpreted as I-waves (Fehlings et al. 1988). It is therefore likely that MEPs observed in this study are also produced by a combination of D- and I-waves. Further experiments with direct recording of descending volleys in spinal cord in response to TMS are necessary to clarify this issue.

The motor threshold (MT) concept is essential to human TMS, especially in paradigms that compare two TMS assessments. The motor threshold is thought to reflect the membrane excitability of pyramidal cells (Cracco et al. 1999). Membrane excitability is affected by several factors in our model particularly by propofol. Our results reveal that MT is a stable parameter without significant intraindividual fluctuation over 4 h of propofol administration. The measure confirms the stability of motor excitability during this period. Additionally, MT allows for interindividual correction of experimental factors that influence motor excitability, such as coil-position and coil-cortex distance (Woodforth et al. 1999).

Limitations

The limitation of TMS in rodents using commercially available equipment is poor focality of stimulation. This feature is clearly demonstrated in our study by the presence of two morphologies of MEP_{TMS} attributable to different neuronal generators. Focality is not needed as long as the overall corticospinal excitability is to be measured. Somatotopic mapping of motor cortex cannot be achieved until smaller coil designs are widely available (Wang et al. 1996).

Conclusions

This study demonstrates the feasibility of TMS in the rat using commercially available TMS equipment. We presented a methodology for reproducible assessment of motor excitability in the propofol-anesthetized rat. Latency differences between MEP to TMS and to cervical electrical stimulation suggest a supraspinal origin of MEP_{TMS}. We identified two types of MEP_{TMS}, for which we assume a cortical and subcortical origin. The sigmoid recruitment curve of $MEP_{TMS,\,1}$ suggests input-output properties similar to those of the human corticospinal system. Rodent TMS methodology is applicable to various models of brain injury and to assessment of motor recovery.

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