A new measure of cortical inhibition by mechanomyography and paired-pulse transcranial magnetic stimulation in unanesthetized rats


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Hsieh T-H, Dhamne SC, Chen J-J, Pascual-Leone A, Jensen FE, Rotenberg A. A new measure of cortical inhibition by mechanomyography and paired-pulse transcranial magnetic stimulation in unanesthetized rats. J Neurophysiol 107: 966–972, 2012. First published October 19, 2011; doi:10.1152/jn.00690.2011.—Paired-pulse transcranial magnetic stimulation (ppTMS) is a safe and noninvasive tool for measuring cortical inhibition in humans, particularly in patients with disorders of cortical inhibition such as epilepsy. However, ppTMS protocols in rodent disease models, where mechanistic insight into the ppTMS physiology and into disease processes may be obtained, have been limited due to the requirement for anesthesia and needle electromyography. To eliminate the confounding factor of anesthesia and to approximate human ppTMS protocols in awake rats, we adapted the mechanomyogram (MMG) method to investigate the ppTMS inhibitory phenomenon in awake rats and then applied differential pharmacology to test the hypothesis that long-interval cortical inhibition is mediated by the GABAA receptor. Bilateral hindlimb-evoked MMGs were elicited in awake rats by long-interval ppTMS protocols with 50-, 100-, and 200-ms interstimulus intervals. Acute changes in ppTMS-MMG were measured before and after intraperitoneal injections of saline, the GABAA agonist pentobarbital (PB), and GABAA antagonist pentylenetetrazole (PTZ). An evoked MMG was obtained in 100% of animals by single-pulse stimulation, and ppTMS resulted in predictable inhibition of the test-evoked MMG. With increasing TMS intensity, MMG amplitudes increased in proportion to machine output to produce reliable input-output curves. Simultaneous recordings of electromyography and MMG showed a predictable latency discrepancy between the motor-evoked potential and the evoked MMG (7.55 ± 0.08 and 9.16 ± 0.14 ms, respectively). With pharmacological testing, time course observations showed that ppTMS-MMG inhibition was acutely reduced following PTZ (P < 0.05), acutely enhanced after PB (P < 0.01) injection, and then recovered to pretreatment baseline after 1 h. Our data support the application of the ppTMS-MMG technique for measuring the cortical excitability in awake rats and provide the evidence that GABAA receptor contributes to long-interval paired-pulse cortical inhibition. Thus ppTMS-MMG appears a well-tolerated biomarker for measuring GABAA-mediated cortical inhibition in rats.

long-interval intracortical inhibition; mechanomyogram; GABA

TRANSCRANIAL MAGNETIC STIMULATION (TMS) is a well-tolerated technique for measuring regional cortical excitability that is gaining acceptance as a means to examine cortical physiology in healthy human subjects and in patients with neurological disorders such as epilepsy, stroke, and mild concussion (Manganott et al. 2001, 2008; Tremblay et al. 2011; Valzania et al. 1999). In TMS, the cortex is activated by a powerful fluctuating extracranial magnetic field, which induces small intracranial electrical currents (reviewed in Kobayashi and Pascual-Leone 2003). In humans, TMS can be applied to the motor cortex and is commonly coupled with surface electromyography (EMG) for quantifying the motor-evoked potential (MEP) that is produced in the hand contralateral to the stimulation site (Pascual-Leone et al. 1998).

For purposes of measuring cortical inhibition, pairs of stimuli are delivered in a protocol termed paired-pulse TMS (ppTMS) such that each successive test stimulus is preceded by a conditioning stimulus, and the two stimuli are separated by a fixed interstimulus interval (ISI; Chen 2004; Chen et al. 1998; Ziemann et al. 1996). Relevant to the present report, long (50–300 ms) ISI ppTMS, delivered over the motor cortex, leads to a predictable suppression of the MEP that is produced by the second (test) stimulus, likely due to a GABA-mediated regional inhibition of the cortical response triggered by the second of the two stimuli. This process, referred to as long-interval intracortical inhibition (LICI), is abnormal in patients with neurological disorders such as epilepsy (Badawy et al. 2010 a, b).

To enable translational research in rat disease models, we recently adapted ppTMS methods to anesthetized rats and identified that a LICI-type phenomenon [in rodents, termed long-interval ppTMS (LI-ppTMS) inhibition] is present and preserved under anesthesia condition, showing that a loss of inhibition can be detected in an acute chemoconvulsant rat seizure model (Vahabzadeh-Hagh et al. 2011). However the requirement for anesthesia in rat ppTMS presents a number of problems: 1) it distinguishes rat from human ppTMS protocols where no anesthesia is required; 2) it is a confounding factor for interpreting the pharmacology of cortical inhibition; and 3) depending on the anesthetic choice and choice of animal model, general anesthesia may be either injurious or neuroprotective and thus may alter cortical physiology, particularly rodent models of brain injury. Accordingly, we developed novel methods for ppTMS in unanesthetized rats that rely on the mechanomyogram (MMG; Reza et al. 2005), a technique where motor cortex activation is detected and quantified by
limb accelerometry rather than needle EMG where anesthesia is required.

Here, we exploit the evoked MMG method to provide mechanistic insight into the ppTMS inhibitory phenomenon. Specifically, we confirm that ppTMS-MMG protocols enable a measure of cortical inhibition and apply differential pharmacology to test the hypothesis that LI-ppTMS inhibition is mediated by the GABA$_A$ receptor in the rat without anesthesia as a confounding factor.

**METHODS**

*Animals.* Thirty-four adult male Long-Evans rats (485 ± 50 g) were used for the current experiment. Animals were housed in standard cages at a constant temperature control with a 12:12-h light-dark cycle and had continuous water and food before the experimental procedures. All animal procedures were approved by the Animal Care and Use Committee at Children’s Hospital (Boston, MA) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Mechanomyography. To enable ppTMS measures of cortical inhibition in awake rats, we adopted the MMG, a noninvasive measurement of muscle movement, to unanesthetized rats. Acceleration of the foot associated with motor cortex stimulation was recorded by a miniature three-axis accelerometer with an embedded amplifier (15 × 13 × 2 mm; 1 g; sensitivity: 0.8 V/g; AGB3V2; Asakusa Giken). After ~15-s exposure to inhaled isoflurane, accelerometers were rapidly and transiently secured with adhesive tape to the bilateral plantar foot surface (Fig. 1). Rats awoke ~30 s after electrode placement and remained awake for the duration of the experiment. The MMG signal was digitized at 1 kHz, band-pass filtered 1–250 Hz, and stored for further offline analysis (PowerLab 8/30; ADInstruments). Each individual evoked MMG signal was measured as the three-vector sum of peak-to-peak voltage output from the accelerometer. To determine the relationship between MMG response and stimulus intensity, the MMG input-output curve was generated by systematically adjusting the stimulator intensity in steps of 10% machine output (MO) from 60% to 100% with a 7-s interpulse interval for each of five TMS intensities. Ten peak-to-peak evoked MMG amplitudes of the same TMS intensity were averaged.

**EMG and electromechanical coupling measurement.** To assess electromechanical coupling between the evoked MMG and the MEP induced by TMS, one group of rats ($n = 7$) were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). The MEP and evoked MMG were simultaneously recorded by tibialis anterior needle EMG and footpad accelerometry. The EMG signal sampled at 10 kHz was band-pass filtered at cutoff frequencies of 100–1,000 Hz and amplified by 1,000 times (A-M Systems Model 1700; Sequim, WA; Rotenberg et al. 2010; Vahabzadeh-Haghi et al. 2011). We then measured the onset latency of both the mechanical and electrical signals, determined as the signal amplitude > 3 SD above the average baseline signal noise collected 1 s before the TMS artifact.

**ppTMS.** All ppTMS sessions were performed with a Magstim Rapid magnetic stimulator (Whitland, Carmarthenshire, Wales, United Kingdom) and a figure-of-eight coil (outside diameter = 66 mm, inside diameter = 15 mm; double small coil; Magstim). The coil was held in the stereotaxic frame and positioned in the midline at the interocular line over the dorsal scalp, a position that reliably elicits bilateral hindlimb movement and bilateral hindlimb MMG (Fig. 1). Pairs of stimuli were delivered with a 7-s interpulse interval for all conditions. The magnitude of paired-pulse-evoked MMG inhibition was calculated as the ratio of the first (conditioning) evoked MMG to the second (test) evoked MMG at each test intensity (60, 70, 80, 90, and 100% MO) and at three separate ISIs (50, 100, and 200 ms).

**Drug treatment.** To evaluate the contribution of cortical GABA$_A$ receptors on ppTMS measures of cortical inhibition in awake rats, we divided 27 rats into 3 equal groups to receive intraperitoneal 0.9% saline (1 ml/kg) for sham control group, the GABA$_A$ agonist pentobarbital (PB; 25 mg/kg), and the GABA$_A$ antagonist pentylentetrazole (PTZ; 25 mg/kg). The PB and PTZ doses were subanesthetic and subconvulsive, respectively. The evoked MMG input-output curve and paired-pulse MMG inhibition at 50, 100, and 200 ms within 60–100% MO were obtained before drug or saline injection, 10 min after injection, and 60 min after injection.

Data processing and statistical analysis. Data were analyzed using SPSS version 17.0 with significance level defined as $P < 0.05$ for each assessment. All data are presented as means ± SE. Paired $t$-test was used to examine the means of duration between the MEP and the evoked MMG. Paired-pulse inhibition was expressed as ratio (percentage) of the conditioned evoked MMG to unconditioned evoked MMG, per ISI, per rat.

To determine the effect of TMS intensity within 60–100% MO range on paired-pulse MMG inhibition, one-way ANOVA was used to compare the inhibition levels each stimulus intensity followed by a Bonferroni post hoc test before drug treatment. One-way ANOVA with Bonferroni posttests was also performed to determine separately the contributions of individual ISIs in measures of long-interval paired-pulse inhibition. The effects on the
unconditioned evoked MMG following drug injection were evaluated by one-way repeated-measures ANOVA with time as within-subject main factor. For the changes in LI-ppTMS for multiple ISIs after drug injection, two-way repeated-measures ANOVA was used to evaluate separately the contributions of time, drug choice, and their interaction. Bonferroni posttests were performed to compare groups per ISI at each time point when the main effect of group was significant.

RESULTS

TMS-MMG feasibility. The evoked MMG was successfully recorded in 100% (34 of 34) of rats. The TMS-MMG signal, as the more conventional TMS-EMG, enabled the generation of input-output curve with progressive increase of TMS intensity. Figure 2A shows representative captures of the MMG signal elicited by single-pulse TMS. The obtained input-output curve (averaged from 27 rats; Fig. 2B) shows a consistent slope and progressive increase in evoked MMG amplitude as TMS intensity is increased from 60 to 100% MO, similar to our previously published evoked brachioradialis MEP recruitment curve (Rotenberg et al. 2010). We note that the MMG summed voltage is equal in the left and right hindlimb, confirming symmetric activation with a midline TMS coil position.

Simultaneous MEP and evoked MMG in rats. With stimulus intensity of 80–100% MO, both EMG and MMG responses to single-pulse TMS were recorded in seven rats. Representative traces in EMG and MMG during single-pulse TMS are shown in Fig. 3A. Figure 3B illustrates the average of latency changes in EMG and MMG. The average latency shows 7.55 ± 0.08 ms in EMG and 9.16 ± 0.14 ms in MMG, respectively. A significant and consistent difference was observed between each latency (t = −9.19, P < 0.001), which confirms electromechanical coupling between the EMG and MMG signals (Fig. 3B).

LI-ppTMS and evoked MMG inhibition in awake rats. With ppTMS protocol applied to an awake rat, the evoked MMG demonstrated reliable inhibition of the test stimulus. Examples from individual responses during single- and ppTMS at 50-, 100-, and 200-ms ISI are displayed in Fig. 4A. Compared with unconditioned (1st of the pair) evoked MMG, LI-ppTMS revealed significant inhibition at all ISIs with maximal inhibition at ISI of 100 ms.

With regard to the level of ppTMS inhibition as a function of stimulus intensity, a one-way ANOVA reveals significant effects of intensity in both limbs at all tested (50-, 100-, and 200-ms) ISI (all P < 0.001). For post hoc comparisons (Fig. 4B), ppTMS inhibition was enhanced with increasing stimulus intensity. Compared with the inhibition level at 60% MO, the ppTMS increased significantly as reflected in a progressively smaller test-to-conditioning evoked MMG ratio.

Comparison of LI-ppTMS evoked MMG inhibition under three ISIs using one-way ANOVA reveals a significant effect of the ISI (F₂,78 = 39.13, P < 0.001 in left hindpaw; F₂,78 = 43.17, P < 0.001 in right hindpaw). These data support strict dependence of the degree of inhibition on ISI, revealing that LI-ppTMS inhibited the conditioned evoked MMG by 63.0 ± 1.8% at 50-ms ISI, 42.2 ± 1.3% at 100-ms ISI, and 51.2 ± 1.4% at 200-ms ISI (Fig. 4C).
**GABA_\text{A}** contribution to LI-ppTMS-MMG inhibition. To test whether LI-ppTMS inhibition of the evoked MMG in awake rats is mediated by GABA_\text{A} receptor activation, rats (n = 27) were administered PB, PTZ, or saline as the control condition. The unconditioned MMG was not affected by the treatment and did not change significantly over time (Fig. 5; \( F_{2,16} = 0.19, P = 0.828 \) in left; \( F_{2,16} = 0.12, P = 0.888 \) right), time effect (\( F_{2,16} = 0.4, P = 0.677 \) in left; \( F_{2,16} = 0.15, P = 0.864 \) in right), or interaction effect (\( F_{4,32} = 0.12, P = 0.976 \) in left; \( F_{4,32} = 0.08, P = 0.989 \) in right). However, ppTMS-MMG measures of cortical inhibition were reliably affected by both drugs. Altered ppTMS inhibition after saline, PB, or PTZ injection is illustrated in Fig. 6, which displays representative tracings at three time periods: preinjection, 10 min after injection, and 60 min after injection.

In support of our overall hypothesis and consistent with our earlier experiments in anesthetized rats (Vahabzadeh-Hagh et al. 2011), LI-ppTMS inhibition was reduced by GABA_\text{A} antagonism with PTZ and enhanced by GABA_\text{A} agonism with PB injection. Two-factor ANOVA for treatment and time demonstrated a significant time \times group interaction in left limb (\( F_{4,32} = 8.31, P < 0.001 \) at 50-ms ISI; \( F_{4,32} = 8.83, P < 0.001 \) at 100-ms ISI; \( F_{4,32} = 9.19, P < 0.001 \) at 200-ms ISI) and in right limb (\( F_{4,32} = 9.64, P < 0.001 \) at 50-ms ISI; \( F_{4,32} = 9.47, P < 0.001 \) at 100-ms ISI; \( F_{4,32} = 15.13, P < 0.001 \) at 200-ms ISI), significant intergroup differences for LI-ppTMS percent inhibition in left side (\( F_{2,16} = 5.58, P = 0.014 \) at 50-ms ISI; \( F_{2,16} = 6.54, P = 0.008 \) at 100-ms ISI; \( F_{2,16} = 4.73, P = 0.024 \) at 200-ms ISI) and in right limb (\( F_{2,16} = 6.24, P = 0.015 \) at 50-ms ISI; \( F_{2,16} = 5.50, P = 0.015 \) at 100-ms ISI; \( F_{2,16} = 3.98, P = 0.04 \) at 200-ms ISI) but not in time factor in left limb (\( F_{2,16} = 2.73, P = 0.095 \) at 50-ms ISI; \( F_{2,16} = 1.87, P = 0.186 \) at 100-ms ISI; \( F_{2,16} = 3.60, P = 0.051 \) at 200-ms ISI) or in right limb (\( F_{2,16} = 1.45, P = 0.264 \); \( F_{2,16} = 0.51, P = 0.608 \) at 100-ms ISI; \( F_{2,16} = 1.55, P = 0.243 \) at 200-ms ISI). Bonferroni posttests demonstrate that this difference was largely driven by the significant PB and PTZ treatment effects observed at 10

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**Fig. 4.** Long-interval paired-pulse inhibition of the test evoked MMG as a function of 3 interstimulus intervals (ISIs) in rats. 

A. representative data during single- (spTMS; left) or paired-pulse TMS (ppTMS; right) within each ISI of 50, 100, and 200 ms, respectively. Arrow indicates the onset of the test stimulus. 

B. effect of a stimulus intensity on paired-pulse inhibition at each of 3 ISIs. Note the more prominent inhibition while increasing conditioning stimulus. Asterisks represent significant differences compared with inhibition at 60% MO by Bonferroni post hoc test. 

C. the graph shows the conditioned MMG peak-to-peak amplitude normalized to unconditioned MMG peak-to-peak amplitude, expressed as the percentage of unconditioned MMG. *P < 0.05; **P < 0.01; ***P < 0.001 per ISI pair comparison by Bonferroni post hoc test. Data are expressed as means ± SE.

**Fig. 5.** Changes in unconditioned MMG over time following saline, pentobarbital (PB), or pentylenetetrazole (PTZ) administration. Graph shows the change in unconditioned MMG as a percentage of preinjection MMG (means ± SE). The measured parameters were compared with preinjection level in each stage. No significant differences were found when compared with preinjection value. Pre, before drug treatment; P10, 10 min postinjection; P60, 60 min postinjection.
min postinjection at all tested ISI \((P < 0.05)\) but not significant at 1-h postinjection when compared with saline group (Fig. 7).

**DISCUSSION**

We report for the first time the feasibility of a quantified measure of cortical excitability and cortical inhibition by single-pulse TMS and by ppTMS in rats without the need for anesthesia. These measures were enabled by the MMG, which we demonstrated is sufficiently sensitive to record both the magnitude of corticospinal activation and the extent of intracortical inhibition as supported by the generation of the evoked MMG input-output curve and the paired-pulse-evoked MMG inhibition profile, respectively.

TMS-MMG methods have been described in humans shown to be highly correlated to the MEP (Reza et al. 2005). In the present study, we find analogous results in rats. The TMS-MMG protocol was well-tolerated by all animals. Once fully alert, in our experience, each rat tolerated the torso restraint for \(\sim 5\) min, which allowed time for a sufficient number of TMS-MMG trials either to generate an input-output curve to measure cortical excitability or to obtain a measure of paired-pulse inhibition. Furthermore, the electromechanical coupling of the evoked MMG and the MEP in anesthetized rats suggests that in the special circumstances of awake rat TMS, the MMG is an adequate substitute for the EMG to provide unique information during TMS. Additionally, the MEP latency suggests that the signal is of cortical origin. By extension, we assume the evoked MMG that follows the MEP is also of cortical origin, although we recognize that further studies in rodent will be necessary to clarify the origin of any TMS signal (Luft et al. 2001; Rotenberg et al. 2010). Thus, taken together, these data imply the potential utility of TMS-MMG in future studies aimed to measure the inhibition-to-excitation ratio either in rodent disease models or in animals exposed to a specific pharmacological or electrophysiological manipulation.

We note that without anesthesia, the TMS-MMG protocol in ways more closely approximates human experimental TMS than prior rat TMS methods (Luft et al. 2001; Rotenberg et al. 2010; Vahabzadeh-Hagh et al. 2011). For instance, the noninvasive nature of the MMG in rodents approximates noninvasive surface EMG in humans and should enable longitudinal followup of an experimental
manipulation by repeated studies in individual rodent subjects. Such sequential recording would be compromised by repeated needle insertion onto a small muscle of rats and repeated exposure to anesthetic. Thus we anticipate the practical application of the above-described methods in tracking long-term effects of an experimental manipulation (such as brain injury) on GABA-mediated cortical inhibition.

In the present experiment, we applied the ppTMS-MMG protocol to test the dependence of LI-ppTMS inhibition on the GABA_A receptor. In a prior experiment, we found that LI-ppTMS inhibition in anesthetized rats was reduced after exposure to a convulsive PTZ dose (Vahabzadeh-Hagh et al. 2011). However, whether this loss of inhibition was due to GABA_A receptor antagonism or to seizure was not apparent in the prior study. Here, without anesthesia, ppTMS-MMG enables detection of either inhibition gain or inhibition loss with doses of a GABA_A agonist (PB) or antagonist (PTZ) that are subanesthetic and subconvulsive, respectively. Thus our finding of inhibition gain with low-dose PB and inhibition loss with low-dose PTZ confirm the critical contribution of the GABA_A receptor to the long-interval cortical inhibition phenomenon.

**Conclusion and practical significance.** Our data are an advance in translational TMS methods aimed to approximate human TMS protocols in rats. With TMS-MMG, as with TMS-EMG in humans, measures of cortical excitability may be obtained rapidly and safely in awake rodents. One practical application for this technique may be in experiments where rapid and sequential cortical inhibition measures may be desired, as demonstrated in sequential measures obtained at intervals after either PB or PTZ injection in our study. Similar serial measures could be of particular importance in a number of rat models of human neurological disease such as epilepsy and traumatic brain injury where the state of cortical inhibition may fluctuate with time. Without the confounding effects of anesthesia, we also anticipate the TMS-MMG method to be of use in studies aimed at drug discovery where relatively subtle and dose-dependent effects of a pharmaceutical agent on cortical inhibition may be investigated.

The TMS-MMG rodent embodiment of human TMS protocols also enables the improved insight into the basic mechanisms of cortical inhibition and excitation, since confounding effects of anesthesia are avoided. In the present case, we demonstrate the potential contribution of the GABA_A receptor to LICI by GABA_A agonism and antagonism with selected agents. Although beyond the scope of this study, we anticipate future experiments aimed to test the contributions of a range of pharmacological and nonpharmacological interventions to cortical inhibition in healthy rodents and in rodent disease models.

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**Fig. 7.** Changes in LI-ppTMS inhibition after saline, PB, and PTZ administration. Data were compared with the average (Ave) level of inhibition in the saline (control) group at each time point for 50-ms ISI (A), 100-ms ISI (B), and 200-ms ISI (C). Note clear separation between PB and PTZ, with PTZ causing reduced inhibition for all time points and the PB causing increased inhibition after 10-min administration (P10) but return to normal inhibition 1 h postinjection (P60). Asterisks represent significant differences compared with saline group at specific time points (unpaired t-test); *P < 0.05, **P < 0.01, ***P < 0.001. L+R, left and right hindpaw averaged values.
DISCLOSURES
A. Pascual-Leone serves on advisory boards for Nexstim, Neuronix, Starlab Neuroscience, Allied Mind, Neosync, and Novavision and holds intellectual property on integration of TMS with EEG and MRI.

REFERENCES