A New Measure of Cortical Inhibition by Mechanomyography and Paired-pulse Transcranial Magnetic Stimulation in Unanesthetized Rats

Running Head: MMG and paired-pulse TMS in Rats

Tsung-Hsun Hsieh\textsuperscript{1,2}, Sameer C. Dhamne\textsuperscript{1}, Jia-Jin J Chen\textsuperscript{2}, Alvaro Pascual-Leone\textsuperscript{3,4}, Frances E. Jensen\textsuperscript{1,5}, Alexander Rotenberg\textsuperscript{1,3*}

\textsuperscript{1} Department of Neurology, Children’s Hospital, Harvard Medical School, Boston, MA 02115, USA
\textsuperscript{2} Department of Biomedical Engineering, National Cheng Kung University, Tainan 701, Taiwan
\textsuperscript{3} Berenson Allen Center for Noninvasive Brain Stimulation, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA
\textsuperscript{4} Institut Universitari de Neurorehabilitació Guttmann, Universidad Autónoma de Barcelona, Badalona, Spain.
\textsuperscript{5} Program in Neurobiology, Harvard Medical School, Boston, MA 02115, USA

* Corresponding Author: Alexander Rotenberg, M.D., Ph.D.; Dept. Neurology, Children’s Hospital, 300 Longwood Avenue, Boston, MA 02215.
Tel.: +1 617 355 8071; Fax: +1 617 730 0463;
E-mail: alexander.rotenberg@childrens.harvard.edu

Figures: 6
Tables: 0
Abstract:
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 GABA_A 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 GABA_A agonist pentobarbital (PB) and GABA_A 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 ms and 9.16±0.14 ms, respectively). With pharmacologic 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 one hour. Our data support the application of the ppTMS-MMG technique for measuring the cortical
excitability in awake rats and provide the evidence that GABA<sub>A</sub> receptor contributes
to long-interval cortical inhibition. Thus ppTMS-MMG appears a well-tolerated
biomarker for measuring GABA<sub>A</sub>-mediated cortical inhibition in rats.

**Keywords:** Transcranial magnetic stimulation (TMS), Long interval intracortical
inhibition, Mechanomyogram, GABA
Introduction

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 (Manganotti et al. 2008; Manganotti et al. 2001; 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 (Kobayashi and Pascual-Leone 2003; Pascual-Leone et al. 1998). 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.

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 γ-aminobutyric acid (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 patient with neurological disorders such as epilepsy (Badawy et al. 2010a; Badawy et al. 2010b).

To enable translational research in rat disease models, we recently adapted ppTMS methods to anesthetized rats, identified that a LICI-type phenomenon (in
rodents, termed long-interval 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, requirement for anesthesia in rat ppTMS presents a number of problems; for example: (1) it does not replicate human ppTMS protocols where no anesthesia is required, (2) anesthesia 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 by 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 LICI is mediated by the GABA\textsubscript{A} receptor in the rat without anesthesia as a confounding factor.

**Methods**

**Animals**

Thirty-four adult male Long-Evans rats (485±50g) 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 guidelines of
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 unanaesthetized rats. Acceleration of the foot associated with motor cortex stimulation was recorded by a miniature 3-axis accelerometer with an embedded amplifier (AGB3V2, Asakusa Giken, Japan; 15 x 13 x 2 mm; 1 g; sensitivity: 0.8 V/G). After approximately 15 seconds exposure to inhaled isoflurane, accelerometers were rapidly and transiently secured with adhesive tape to the bilateral plantar foot surface (Fig.1). Rats awoke approximately 30 seconds 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, Australia). Each individual evoked MMG signal was measured as the 3-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 sec inter-pulse interval for each of five TMS intensities. Ten peak-to-peak evoked MMG amplitudes of the same TMS intensity were averaged.

**Electromyography 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 foot pad accelerometry. The EMG signal sampled at 10 kHz was bandpass filtered at cut-off frequencies of 100–1000 Hz and amplified by 1,000 times (AM Systems Model 1700; Sequim, WA) (Rotenberg et al. 2010; Vahabzadeh-Hagh et al. 2011). We then measured the onset latency of both the mechanical and electrical signals, determined as the signal amplitude greater than three standard deviations above the average baseline signal noise collected one sec prior to the TMS artifact.

**Paired-pulse transcranial magnetic stimulation**

All ppTMS sessions were performed with a Magstim Rapid magnetic stimulator (Magstim Co., Whitland, Carmarthenshire, Wales, UK) and a figure of eight coil (outside diameter = 66 mm, inside diameter = 15 mm; Double small Coil, Magstim, UK). The coil was held in the stereotaxic frame and positioned in the midline at the interocular line over the dorsal scalp, a position which reliably elicits bilateral hindlimb movement and bilateral hindlimb MMG (Fig.1). Pairs of stimuli were delivered with a 7 sec inter-pair 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 ms, 100 ms, and 200 ms).

**Drug treatment**

To evaluate the contribution of cortical GABA<sub>A</sub> receptors on ppTMS measures of cortical inhibition in awake rats, we divided 27 rats into three 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 pentylenetetrazole
(PTZ; 25 mg/kg). The PB and PTZ doses were sub-anesthetic and subconvulsive,
respectively. The evoked MMG input-output curve and paired-pulse MMG inhibition
at 50 ms, 100 ms and 200 ms within 60%-100% MO were obtained before drug or
saline injection, 10 minutes after injection, and 60 minutes after injection.

**Data processing and statistical analysis**

Data were analyzed using SPSS version 17.0 (SPSS Inc., USA) with significance
level defined as $p < 0.05$ for each assessment. All data were presented as average ±
standard error of the mean (SEM). Paired t-test was used to examine the means of
duration between the MEP and the evoked MMG. Paired-pulse inhibition was
expressed as percentage of the conditioned evoked MMG to unconditioned evoked
MMG, per ISI, per rat.

To determine the effect of intensity effect within 60%-100% MO range on
paired-pulse MMG inhibition, one-way ANOVA was used to compare the inhibition
levels at each stimulus intensity followed by a Bonferroni post hoc test at pre-durg
treatment. One-way ANOVA with Bonferroni post-tests 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 measure of ANOVA with time as
within-subject main factor. For the changes in long-interval paired-pulse TMS
(LI-ppTMS) for multiples ISIs after drug injection, two-way repeated measures of
ANOVA was used to evaluate separately the contributions of time, drug choice and
their interaction. Bonferroni post-tests were performed to compare groups per ISI at
Results

TMS-MMG feasibility

The evoked MMG was successfully recorded in 100% (34 of 34) 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 7 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, but 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 pp-TMS at 50 ms, 100 ms, and 200 ms ISI are displayed in Figure 4A. Compared to unconditioned (first 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 ms, 100 ms and 200 ms) ISI (all $p<0.001$). For post hoc comparisons (Figure 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: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 ($F_{2,78}=39.13$, $p<0.001$ in left hindpaw; $F_{2,78}=43.17$, $p<0.001$ 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	extsubscript{A} contribution to LI-ppTMS-MMG inhibition

To test whether LI-ppTMS inhibition of the evoked MMG in awake rats is mediated by GABA	extsubscript{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 in treatment effect ($F_{2,16}=0.19$, $p=0.828$ in left; $F_{2,16}=0.12$, $p=0.888$ in right); time effect ($F_{2,16}=0.4$, $p=0.677$ in left; $F_{2,16}=0.15$, $p=0.864$ in right); 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 pp-TMS inhibition after saline, PB or PTZ injection is illustrated in Figure 5A which displays representative tracings at three time periods: pre-injection, 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 A antagonism with PTZ and enhanced by GABA A agonism with PB injection (Fig 5B). Two-factor ANOVA for treatment and time demonstrated a significant time × group interaction in left limb (F4,32=8.31, P<0.001 at 50ms ISI; F4,32=8.83, P<0.001 at 100ms ISI; F4,32=9.19, P<0.001 at 200 ms ISI) and in right limb (F4,32=9.64, P<0.001 at 50ms ISI; F4,32=9.47, P<0.001 at 100ms ISI; F4,32=15.13, P<0.001 at 200ms ISI), significant intergroup differences for LI-ppTMS percent inhibition in left side (F2,16=5.58; p=0.014 at 50ms ISI; F2,16=6.54; p=0.008 at 100ms ISI; F2,16=4.73; P=0.024 at 200 ms ISI) and in right limb (F2,16=6.04; P=0.01 at 50ms ISI; F2,16=5.50; P=0.015 at 100ms ISI; F2,16=3.98; P=0.04 at 200ms ISI), but not in time factor in left limb (F2,16=2.73, P=0.095 at 50ms ISI; F2,16=1.87, P=0.186 at 100ms ISI; F2,16=3.60, P=0.051 at 200 ms ISI) and in right limb (F2,16=1.45, P=0.264; F2,16=0.51, P=0.608 at 100ms ISI; F2,16=1.55, P=0.243 at 200ms ISI). Bonferroni post-tests demonstrate that this difference was largely driven by the significant PB and PTZ treatment effects observed at 10 min post injection at all tested ISI (p<0.05) but not significant at 1 hour post injection when compared with saline group (Fig. 6).

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 approximately five
minutes, which allowed time for a sufficient number of TMS-MMG trials to either
generate an input-output curve to measure cortical excitability, or to obtain a measure
of paired-pulse inhibition. Further, 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 is the evoked MMG which follows the MEP
is as well 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: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 follow-up of an experimental manipulation by repeated studies in individual rodent subjects. Such sequential recording would be compromised by repeated needle insertion onto a small rat muscle 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<sub>A</sub> 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<sub>A</sub> 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<sub>A</sub> agonist (PB) or antagonist (PTZ) that are subanesthetic and subconvulsant, 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<sub>A</sub> 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
neurologic 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
potential confounding effects of anesthesia are avoided. In the present case, we
demonstrate the contribution of the GABA<sub>A</sub> receptor to LICI by selective GABA<sub>A</sub>
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
pharmacologic and nonpharmacologic interventions to cortical inhibition in healthy
rodents and in rodent disease models.

Acknowledgements and Grants

This work was supported by NIH NINDS K08 NS055895 and the Translational
Research Program at Children’s Hospital (Rotenberg). The authors also thank the
National Science Council and the National Health Research Institutes of Taiwan for
financial supports in this work under grants NSC982917I006113 and
NHRI-EX98-9535EI (Hsieh and Chen), and CIMIT (Rotenberg, Pascual-Leone). Dr.
Pascual-Leone serves on the scientific advisory boards for Nexstim, Neuronix, Starlab
Neuroscience, Allied Mind, Neosync, and Novavision, and holds intellectual property
on the real-time integration of transcranial magnetic stimulation (TMS) with
electroencephalography (EEG) and magnetic resonance imaging (MRI). His contribution to this work was also supported by a grant from the National Center for Research Resources: Harvard Clinical and Translational Science Center (UL1 RR025758).
Figure legends

Fig. 1. (A) TMS-MMG setup. Unanesthetized rat is restrained on a platform with four straps with minimal discomfort. The figure-of-eight TMS coil is centered over the dorsal scalp at the interaural line. (B) Example of MMG sensor placement. The MMG is obtained by two 3-axis square-shaped accelerometer elements on each ventral surface of the foot by adhesive tape while the rat is under brief isoflurane anesthesia prior to placement into the restraint.

Fig. 2. (A) Representative evoked MMG from one rat as a function of TMS intensity from 60–100% MO. An increase of MMG amplitude was noted with an increasing level of TMS intensity. (B) The average of input–output curve in unconditioned MMG from 27 rats.

Fig. 3. Representative trace of motor evoked potential (MEP) by EMG recording and evoked MMG during single TMS in one rat is shown (A). Mean latency of MEP and MMG in 7 rats (B). ***p < 0.001 as compared between EMG and MMG.

Fig. 4. Long interval paired-pulse inhibition of the test MMG as a function of three interstimulus intervals (ISIs) in rats. (A) Representative data during single (left) or paired-pulse TMS (right) within each ISIs of 50, 100 and 200 ms, respectively. Arrow indicates the onset of the test stimulus. (B) Effect of a stimulus intensity on of paired-pulse inhibition at each of three ISIs. Note the more prominent inhibition while increasing conditioning stimulus. Asterisks represent significant differences as compared to 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 percent of unconditioned MMG. *p<0.05; **p<0.01; ***p<0.001 compared between two each ISIs with Bonferroni post hoc test. Data are expressed as mean ± SEM.

Fig. 5. Examples of time course changes of LI-ppTMS inhibition at 200ms ISI following saline, PB and PTZ administration. Representative MMG tracings show no obvious change in LI-ppTMS inhibition in the saline group with increased inhibition in PB group and reduced inhibition in the PTZ group (middle column). Note the mild returned LI-ppTMS inhibition after 1 hour PB or PTZ injection (right column). (B) Changes in unconditioned MMG over time following saline, PB or PTZ administration. Graph shows the change in unconditioned MMG as a percent of pre-injection MMG (mean± SEM). The measured parameters were compared with pre-injection level in each stage. No significant differences were found when compared with pre-injection value.

Fig. 6. The changes of LI-ppTMS inhibition after saline, PB and PTZ administration. Data were compared to the average level of inhibition in the saline (control) group at each time point for 50ms ISI (A), 100ms ISI (B) and 200ms ISI (C). Note clear separation between PB and PTZ, with PTZ causing reduced inhibition for all time points, whereas the PB causing increased inhibition after 10 min administration but return to normal inhibition after 1 hour (post) injection. Asterisks represent significant differences compared with saline group at specific time points (unpaired-T test); * p < 0.05, ** p < 0.01, *** p< 0.001.
References


Vahabzadeh-Hagh AM, Muller PA, Pascual-Leone A, Jensen FE, and Rotenberg A. Measures of cortical inhibition by paired-pulse transcranial magnetic stimulation in

