

Mechanisms underlying long-interval cortical inhibition in the human motor cortex: a TMS-EEG study

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Rogasch NC, Daskalakis ZJ, Fitzgerald PB. Mechanisms underlying long-interval cortical inhibition in the human motor cortex: a TMS-EEG study. *J Neurophysiol* 109: 89–98, 2013. First published October 24, 2012; doi:10.1152/jn.00762.2012.—Long-interval cortical inhibition (LICI) refers to suppression of neuronal activity following paired-pulse transcranial magnetic stimulation (TMS) with inter-stimulus intervals (ISIs) between 50 and 200 ms. LICI can be measured either from motor-evoked potentials (MEPs) in small hand muscles or directly from the cortex using concurrent electroencephalography (EEG). However, it remains unclear whether EEG inhibition reflects similar mechanisms to MEP inhibition. Eight healthy participants received single- and paired-pulse TMS (ISI = 100 ms) over the motor cortex. MEPs were measured from a small hand muscle (first dorsal interosseus), whereas early (P30, P60) and late (N100) TMS-evoked cortical potentials (TEPs) were measured over the motor cortex using EEG. Conditioning and test TMS intensities were altered, and modulation of LICI strength was measured using both methods. LICI of MEPs and both P30 and P60 TEPs increased in strength with increasing conditioning intensities and decreased with increasing test intensities. LICI of N100 TEPs remained unchanged across all conditions. In addition, MEP and P30 LICI strength correlated with the slope of the N100 evoked by the conditioning pulse. LICI of early and late TEP components was differentially modulated with altered TMS intensities, suggesting independent underlying mechanisms. LICI of P30 is consistent with inhibition of cortical excitation similar to MEPs, whereas LICI of N100 may reflect presynaptic autoinhibition of inhibitory interneurons. The N100 evoked by the conditioning pulse is consistent with the mechanism responsible for LICI, most likely GABA_B-mediated inhibition of cortical activity.

transcranial magnetic stimulation; electroencephalography; cortical inhibition; motor cortex

CORTICAL INHIBITION REFERS TO suppression of neuronal firing mediated by γ -amino butyric acid (GABA) receptors (Krnjevic 1997). Brain slice studies in animal and human cortical tissue have revealed two main phases of inhibition following stimulation; a “fast” phase mediated by ionotropic GABA_A receptors and a “slow” phase mediated by metabotropic GABA_B receptors (Connors et al. 1988; McCormick 1989). In conscious humans, cortical inhibition can be measured using paired-pulse transcranial magnetic stimulation (TMS; Kujirai et al. 1993; Reis et al. 2008; Valls-Sole et al. 1992), a noninvasive method of stimulating cortical neurons (Barker et al. 1985). When applied at sufficient intensity over the motor cortex, TMS transynaptically activates descending pyramidal corticospinal

neurons resulting in a motor-evoked potential (MEP) in peripheral muscles targeted by the region of cortex stimulated (Amassian et al. 1989; Day et al. 1989). MEPs are easily measured using electromyography (EMG) over the target muscle, and MEP amplitude is considered a basic index of corticospinal excitability (Di Lazzaro et al. 2008). When preceded by a suprathreshold TMS pulse at interstimulus intervals (ISIs) of 50–200 ms, MEP amplitude is significantly reduced and is referred to as long-interval cortical inhibition (LICI; Nakamura et al. 1997; Valls-Sole et al. 1992). LICI is thought to reflect GABA_B-mediated cortical inhibition based on several lines of evidence. First, the time course of LICI corresponds to the slow inhibitory postsynaptic potential measured from animal and human tissue (McCormick 1989; Valls-Sole et al. 1992). Second, LICI is increased following administration of the GABA_B receptor agonist baclofen (Florian et al. 2008; McDonnell et al. 2006). Third, LICI also suppresses a measure of GABA_A-mediated cortical inhibition [short-interval cortical inhibition (SICI); Cash et al. 2010; Chu et al. 2008; Sanger et al. 2001], consistent with GABA_B-mediated presynaptic inhibition of inhibitory interneurons in slice experiments (Davies et al. 1990; Deisz 1999). Finally, the conditioning and test stimulation intensities required for LICI are comparable with *in vitro* studies. LICI requires suprathreshold conditioning intensities (Hammond and Garvey 2006; Valls-Sole et al. 1992), consistent with the high threshold required to activate postsynaptic GABA_B receptors (Connors et al. 1988; Tamas et al. 2003), and LICI is overcome by high-intensity test stimulation (Daskalakis et al. 2002; Sanger et al. 2001), similar to brain slice experiments (Connors et al. 1988).

Measuring LICI with MEP suppression is limited in several important ways. Although there is good evidence that LICI is primarily cortical in origin (Di Lazzaro et al. 2002; Nakamura et al. 1997), inhibition at the level of the spinal cord may also contribute (McNeil et al. 2011). In addition, LICI measures are necessarily limited to the motor cortex due to the dependence of output measures on the motor system. To overcome these limitations, LICI has recently been demonstrated directly from the cortex using TMS combined with electroencephalography (EEG; Daskalakis et al. 2008; Fitzgerald et al. 2008). TMS-EEG is a powerful technique that allows measurement of cortical network properties such as cortical oscillatory frequencies and effective cortical connectivity (Rogasch and Fitzgerald 2012). The amplitude and shape of the TMS-evoked potential (TEP) are dependent on several factors including TMS intensity (Casarotto et al. 2010; Kahkonen et al. 2005; Komssi et al. 2004), site of stimulation (Casarotto et al. 2010;

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Garcia et al. 2011; Kahkonen et al. 2004), and state of the cortical network (Bergmann et al. 2012; Ferrarelli et al. 2010; Massimini et al. 2005; Morishima et al. 2009). In addition, different components of the TEP may convey information on excitatory and inhibitory neural activity (Komssi et al. 2004; Nikulin et al. 2003). For instance, early and late components of the TEP are differentially modulated using TMS paradigms designed to assess cortical mechanisms such as SICI and intracortical facilitation (Ferreri et al. 2011; Paus et al. 2001).

With the use of TMS-EEG, LICI has been measured from both motor and nonmotor regions, such as the prefrontal (Daskalakis et al. 2008) and parietal cortex (Fitzgerald et al. 2008). LICI measured with TMS-EEG is highly reproducible over time (Farzan et al. 2010), specific to the site of stimulation (Daskalakis et al. 2008), and results in inhibition of particular TMS-induced oscillations over different cortical areas (Farzan et al. 2009). Over motor cortex, TEP inhibition peaks at ~100–150 ms following TMS, consistent with GABA_B-mediated inhibition (Fitzgerald et al. 2009a). In addition, LICI measured with EEG correlates with both LICI measured from simultaneous EMG of a small hand muscle and with the cortical silent period, a separate EMG measure related to GABA_B-mediated inhibition (Daskalakis et al. 2008; Farzan et al. 2010). Although LICI measured with EEG appears consistent with EMG measures, it is unclear whether TEP inhibition is sensitive to changes in stimulation parameters. It is also unknown whether inhibition of early and late TEP components is related or independent. Considering that LICI is mediated by inhibitory postsynaptic potentials, information on the precise mechanisms that are targeted by the conditioning stimulus has never been assessed. Of particular interest is the N100, a late TEP component that is thought to directly reflect inhibitory processes (Komssi et al. 2004; Nikulin et al. 2003) and that coincides with delivery of the test pulse in the LICI paradigm.

The aims of this study were twofold. The first aim was to assess if LICI measured over the motor cortex with TMS-EEG is sensitive to changes in both conditioning and test intensities and whether early and late components (i.e., P30, P60, and N100) are modulated dependently or independently with altered stimulation parameters. The second aim was to assess whether the N100 evoked by the conditioning TMS pulse is related to inhibition of the TEPs following the test pulse in the LICI paradigm.

METHODS

Eight volunteers (7 female; 31.5 ± 13 yr old) participated in the current study. Participants were neurologically and mentally healthy, had no family history of epilepsy, and were right handed according to the Edinburgh handedness inventory (laterality quotient mean = 92.5 and range = 70–100; Oldfield 1971). All experimental procedures were approved by the Alfred and Monash Human Research Ethics Committees in accordance with the Declaration of Helsinki, and all participants provided informed written consent before participation.

Experimental arrangement and recordings. Participants were seated comfortably in an arm chair with their right arm resting comfortably in their lap, and their head was supported and their eyes were open throughout the experiment. EMG recordings were obtained from the first dorsal interosseus (FDI) muscle of the right hand using bipolar surface electrodes (Ag/AgCl, 4 mm diameter) placed ~2 cm apart in a belly-tendon montage. A ground electrode common to both recording electrodes was placed over the styloid process of the ulna.

EMG signals were amplified ($\times 1,000$), band pass filtered (high pass = 10 Hz, low pass = 500 Hz), digitized at 2 kHz with a CED interface system (Cambridge Electronic Design), and recorded on a computer for offline analysis.

EEG recordings were obtained from 13 sintered Ag-AgCl, annular electrodes positioned over the left hemisphere in standard 10–20 positions (AF3, F1, F3, F5, FC1, FC3, FC5, C1, C3, C5, CP1, CP3, and CP5). Additional recordings were also obtained from the left and right mastoid process. To monitor eye blinks, electrooculography (EOG) recordings were obtained from four Ag-AgCl electrodes, two located either side of the eyes and two located above and below the left eye. All electrodes were referenced to an electrode located posterior to CZ on the vertex with the exception of the horizontal EOG electrodes, which were referenced to each other. EEG signals were acquired using a Synamps² EEG system (Compumedics). Signals were amplified ($\times 1,000$), low pass filtered (high pass = DC, low pass = 2,000 Hz), digitized at 10 kHz, and recorded on a computer for offline analysis. The high acquisition rate, large operating range, and DC coupling of the EEG amplifier allows recording of the TMS artifact without amplifier saturation, hence permitting recording of TMS-induced TEPs (Daskalakis et al. 2008; Veniero et al. 2009). Impedance levels were kept < 5 k Ω throughout the experiment.

TMS. Biphasic TMS was administered using a figure-of-eight cooled-coil connected to a MagPro R30 stimulator with a MagOption unit (Magventure). The coil was held tangentially over the scalp with the handle pointing backwards and laterally at ~45° to the mid-sagittal line. This coil orientation induces current flow in the underlying cortex in an anterior-posterior direction followed by a posterior-anterior direction (biphasic pulse) and is optimal for eliciting MEPs over motor cortex (Kammer et al. 2001). At the beginning of each session, the coil was moved around the motor cortex and stimulation intensities were gradually increased until the optimal position to elicit MEPs from FDI muscle was found and marked using a felt tip pen. The position was used throughout the remainder of the study and was usually located between C1 and C3. Resting motor threshold (RMT) was then determined as the TMS intensity required to elicit MEPs of > 50 μ V in at least three of five consecutive trials and was expressed as a percentage of maximum stimulator output (%MSO; Rossini et al. 1994). Following assessment of the RMT, the stimulator output was increased until an MEP of ~1 mV was elicited in at least 10 consecutive trials. This TMS intensity (%MSO) was also recorded (S1mV) and used in blocks requiring either a constant conditioning or test TMS intensity. Both RMT and S1mV were determined with the EEG cap and electrodes on. Throughout the study, TMS was delivered randomly at intervals between 0.2 and 0.25 Hz.

Sham stimulation. Sham TMS was administered by positioning the TMS coil at 90° so that the wing of the coil rested on the scalp. With this arrangement, the TMS coil click is audible and some vibrations from the coil discharge are still felt; however, the cortex is not activated by the TMS pulse.

Experimental procedure. Once RMT and S1mV had been determined, the coil was positioned over the motor cortex and held in place using a stand. The coil position was continuously monitored by an experimenter throughout the study and corrected appropriately following any head movement.

LICI was assessed by comparing the test response of single-pulse TMS (unconditioned) to paired-pulse TMS (conditioned). Paired-pulse TMS involved delivering a conditioning pulse 100 ms before the test pulse. This ISI is optimal for MEP inhibition, and previous paired-pulse TMS-EEG studies have shown reliable and reproducible inhibition of EEG both in the motor cortex and other cortical regions (Farzan et al. 2010). Three different conditions were tested: altering conditioning TMS intensities (*condition 1*), altering test TMS intensities (*condition 2*), and sham TMS (*condition 3*). In *condition 1*, TMS was delivered in four blocks of 40 stimuli. *Block 1* consisted of single-pulse TMS alone (S1mV), whereas *blocks 2–4* consisted of paired-pulse TMS with different conditioning intensities in each block

(100, 120, and 140% RMT) and a constant test intensity (S1mV). In *condition 2*, TMS was delivered in six blocks of 40 stimuli. *Blocks 1–3* consisted of single-pulse TMS alone at three different test intensities (110, 125, and 140% RMT), whereas *blocks 4–6* consisted of paired-pulse TMS with constant conditioning intensities (S1mV) and different test intensities (110, 125, and 140% RMT). *Condition 3* consisted of single-pulse sham stimulation. Sham stimulation was delivered in a block of 40 stimuli with an intensity of 140% RMT. For each participant, the condition order was randomized and within each condition the block order was randomized. Participants were given regular breaks between stimulation blocks. To minimize the auditory-evoked potentials resulting from the TMS coil click, white noise was played to participants through inserted earphones for 2 s either side of each TMS pulse (Massimini et al. 2005).

Data analysis. All MEPs containing prestimulus EMG activity (up to 100 ms) were excluded from analysis offline. Peak-to-peak amplitudes of the test MEP were measured from individual trials and the mean test MEP amplitude was calculated for each block of stimulation. For MEPs, LICI strength was calculated by comparing conditioned test responses (paired-pulse) to unconditioned test responses (single-pulse) using *Eq. 1*:

$$\text{LICI}_{\text{MEP}} = (\text{MEP}_{\text{single}} - \text{MEP}_{\text{paired}}) / \text{MEP}_{\text{single}} \times 100 \quad (1)$$

EEG data were analyzed offline using SCAN (Compumedics) and Matlab (MathWorks). Data were downsampled to 1 kHz and then passed through an automated eye-blink correction algorithm (Croft et al. 2005). For each block, EEG signals were segmented around the test TMS pulse (−1,000 to 1,000 ms), baseline corrected with respect to the TMS-free data (−500 to −110 ms before the test TMS pulse), and re-referenced to average mastoids. Data between −5 and 25 ms and −105 and −75 (paired-pulse blocks) were removed using cubic interpolation to eliminate TMS-related artifacts that could contaminate the signal (i.e., TMS artifact, evoked muscle activity, electrode polarization, etc.) (Bergmann et al. 2012; Thut et al. 2011). Signals were then digitally filtered using a zero-phase shift, 1- to 80-Hz band pass filter (48 dB/Oct roll off). At this stage, each epoch was manually inspected and epochs containing TMS artifact, obvious eye blinks, or muscle activity were rejected. Trials rejected from MEP and TEP analysis were cross-referenced so that corresponding trials were rejected in each analysis type (Ilmoniemi and Kicic 2010). Finally, remaining epochs from each trial within a block were averaged.

Where possible, TEPs were measured from electrode C3 as this is generally considered closest to the hand motor area (Fitzgerald et al. 2009b). Excessive line noise and distortion of the TMS artifact was observed in electrodes in which the TMS coil made strong contact. This was typically C1, however, in certain cases both C3 ($n = 2$) and C5 ($n = 2$) also suffered depending on the position of the participant's motor hot spot. In the two participants in which C3 was affected by noise, C5 was used to measure TEPs instead. Previous studies assessing LICI using EEG have focused on inhibition in a period between 50 and 150 ms following the test pulse (Daskalakis et al. 2008). As we were interested in comparing inhibition of early and late components of the TEP, we chose to analyze specific peaks of the TEP instead. Individual peaks and troughs were not consistently observable at the single trial level so analyses of TEPs were performed on averaged data. Latencies of the positive peaks between 25 and 40 ms (P30), 40 and 80 ms (P60), and negative peak between 70 and 130 ms (N100) were determined from the mean trace of each single-pulse block. The analysis periods were chosen as changes in stimulation intensity and coil angle differentially alter the peaks within these ranges, suggesting different underlying mechanisms (Bonato et al. 2006; Komssi et al. 2004). To allow comparisons between unconditioned and conditioned TEPs, the mean amplitude of data ± 5 ms from the single-pulse P30 latency, ± 5 ms from the single-pulse P60 latency, and ± 10 ms from the single-pulse N100 latency was calculated for both single and paired blocks. For *condition 1*, paired-pulse blocks (*blocks 2, 3, and 4*) were compared with the same time windows for the single-pulse

block 1. For *condition 2*, the paired-pulse blocks were compared using the same time windows as single-pulse blocks with corresponding test TMS intensities (*block 1* with *block 4*, etc.). Responses to sham TMS (140% RMT) were compared with the same time window as single-pulse TMS at 110% RMT. For each single-pulse block, TEP signal size was determined by calculating the difference between the maximum and minimum signal within the first 150 ms post TMS and this value was used to normalize TEP inhibition strength. For TEPs, LICI strength was calculated using *Eqs. 2–4*.

$$\text{LICI}_{\text{P30}} = (\text{P30}_{\text{single}} - \text{P30}_{\text{paired}}) / (\text{Max}_{\text{single}} - \text{Min}_{\text{single}}) \times 100 \quad (2)$$

$$\text{LICI}_{\text{P60}} = (\text{P60}_{\text{single}} - \text{P60}_{\text{paired}}) / (\text{Max}_{\text{single}} - \text{Min}_{\text{single}}) \times 100 \quad (3)$$

$$\text{LICI}_{\text{N100}} = (\text{N100}_{\text{single}} - \text{N100}_{\text{paired}}) / (\text{Min}_{\text{single}} - \text{Max}_{\text{single}}) \times 100 \quad (4)$$

Peak analysis of the N100 evoked by the conditioning pulse was precluded by delivery of the test pulse. We therefore adopted an alternative approach and measured the slope of the EEG signal immediately preceding the test TMS pulse. Slope analysis is more typically used in animal experiments to quantify local field potentials following electrical stimulation; however, this approach has recently been adapted to measure cortical excitability in TMS-EEG studies (Huber et al. 2012). Slope was determined by calculating the mean first derivative between −30 and −5 ms, a period that corresponds to the negative slope of the N100 evoked by the conditioning pulse.

For clarity, MEP, P30, P60, and N100 will refer to EMG- and EEG-evoked responses following single-pulse TMS (unconditioned MEPs and TEPs) in the remainder of the text. LICI_{MEP} , LICI_{P30} , LICI_{P60} , and $\text{LICI}_{\text{N100}}$ will refer to the suppression of the aforementioned responses in the paired-pulse paradigm (conditioned MEPs and TEPs), indexed by *Eqs. 1–4*. Conditioning N100 slope will refer to the slope of the N100 evoked by the conditioning TMS pulse in the paired-pulse paradigm.

Statistical analysis. All statistical analysis was performed using SPSS 13.0 for windows (SPSS). In the raw data, significant outliers were detected using a stem and leaf plot and were z-score corrected to 3.29 standard deviations from the mean calculated without outliers. For each dependent variable, normality was tested using a Shapiro-Wilk test. Paired-sample *t*-tests (parametric) or Wilcoxon signed rank tests (nonparametric) were used to compare conditioned with unconditioned MEP and TEP amplitudes in each condition. A Bonferroni correction was applied to correct for multiple comparison. One-way repeated measures analysis of variance (RM-ANOVA; parametric) or Friedman tests (nonparametric) were used to compare the effect of increasing TMS intensity on single-pulse MEPs, TEPs, and conditioning N100 slope and to compare the effect of changing either conditioning intensity (*condition 1*) or test intensity (*condition 2*) on LICI strength. For RM-ANOVAs, Mauchly's test for sphericity was performed and a Greenhouse-Geiser method was used to correct for nonsphericity. Post hoc discrimination of means was performed using either pairwise comparisons (parametric) or Wilcoxon signed rank tests (nonparametric) with Bonferroni corrections for multiple comparisons. Pearson's correlations (parametric) or Spearman's correlations (nonparametric) were used to assess the relationship between LICI strength (MEP and TEP) and N100 conditioning slope separately across conditioning and test intensities. Correlations were also used to assess the relationship between MEP, P30, P60, and N100 amplitudes with increasing single-pulse TMS intensity and between LICI_{MEP} , LICI_{P30} , LICI_{P60} , and $\text{LICI}_{\text{N100}}$ strength across all conditions. Paired-sample *t*-tests (parametric) were used to compare the P30, P60, and N100 amplitudes resulting from single-pulse TMS (110% RMT) with single-pulse sham TMS (140% RMT). Statistical significance was set at $P < 0.05$. Data are mean (SD) in text and Table 1 and means \pm SE in Figs. 1–4.

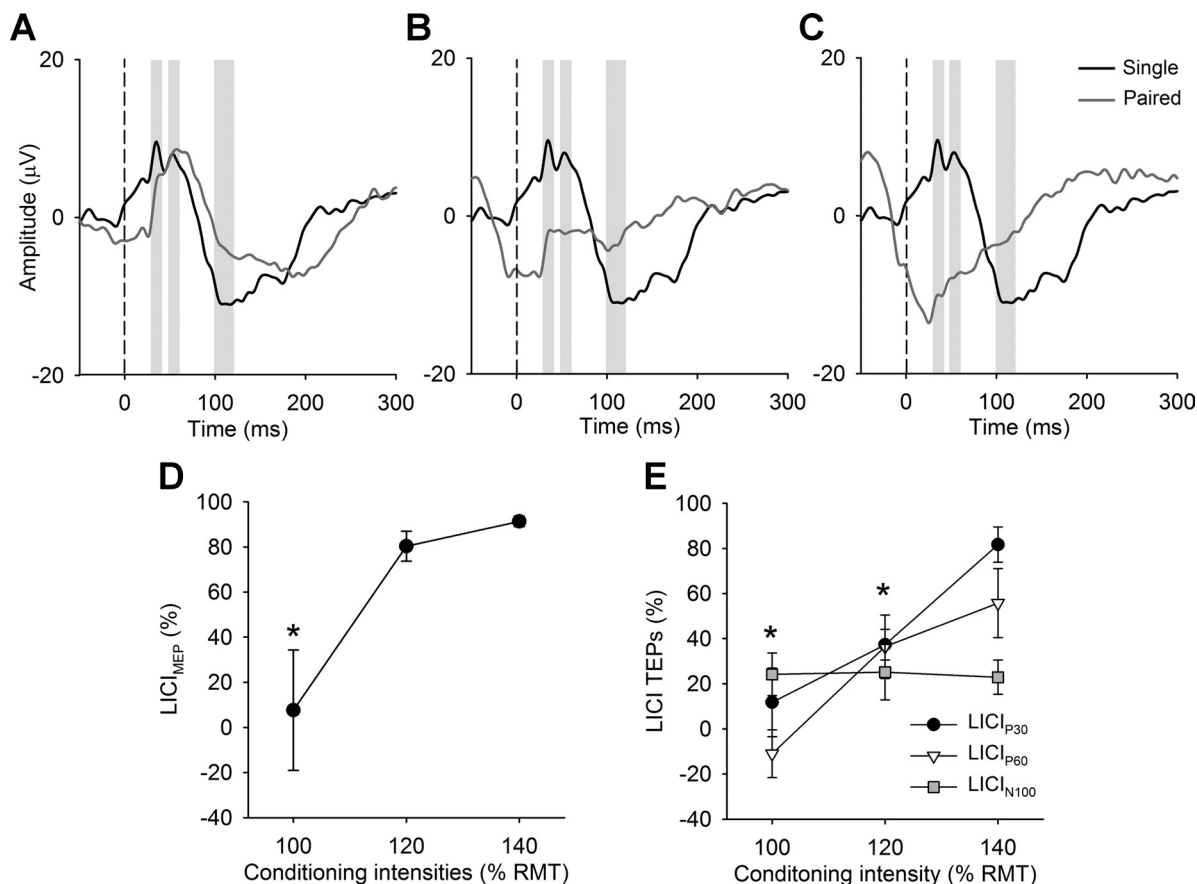


Fig. 1. A–C: group averaged transcranial magnetic stimulation (TMS)-evoked potentials (TEPs) over motor cortex following single (black) and paired (grey) pulses. Conditioning intensities were 100% (A), 120% (B), and 140% (C) of resting motor threshold (RMT) whereas test intensities were constant at S1mV for single- and paired-pulses. Dashed lines indicate timing of the test pulse. Grey boxes mark the average time window used for long-interval cortical inhibition measures (LICI_{P30}, left; LICI_{P60}, middle; and LICI_{N100}, right). N100 evoked by the conditioning pulse can be seen immediately before the test pulse (slope measured from –30 to –5 ms). Note the single-pulse trace is common between A–C. E and F: mean LICI strength measured at different conditioning intensities (100, 120, and 140% RMT) and constant test intensity (S1mV). LICI was measured from motor-evoked potentials (LICI_{MEP}; E) and TEP components (LICI_{P30}, LICI_{P60}, and LICI_{N100}; F). * $P < 0.05$ compared with 140% RMT (LICI_{MEP}, LICI_{P30}, LICI_{P60}).

RESULTS

All participants completed the protocol, and no adverse effects were reported. Mean RMT was $49.6 \pm 12\%$ MSO, and mean S1mV was $59.3 \pm 14\%$ MSO. MEP amplitudes following single-pulse TMS increased with increasing TMS intensity between 110, 125, and 140% RMT ($P = 0.001$). No MEPs were evident during sham stimulation.

Single-pulse TMS resulted in positive peaks in the EEG at ~ 30 and 60 ms and negative troughs at ~ 45 and 100 ms as previously reported (Ilmoniemi and Kicic 2010; Fig. 1, A–C). The P30, P60, and N100 peaks were the most replicable between participants and were used for further analysis. P30 was present in seven of eight participants and statistical tests concerning P30 were performed on this reduced data set. To confirm that bandpass filtering over the interpolated artifact had no effect on P30 amplitude, filtered P30 amplitude was compared with unfiltered P30 amplitude following single-pulse TMS at 110, 125, and 140% RMT. There were no significant differences between filtered and unfiltered P30 amplitudes at any intensity (all $P > 0.4$). P60 was present in six of eight participants, and statistical tests concerning P60 were performed on this reduced data set. N100 was present in all eight participants. P30, P60, and N100 were all significantly greater in amplitude following TMS at 110% RMT than sham TMS at

140% RMT (P30, $P = 0.026$; P60, $P = 0.044$; and N100, $P = 0.007$). Table 1 describes the amplitude and peak latency of P30, P60, and N100 following different TMS intensities. Both amplitude and peak latency of P30 significantly increased with increasing TMS intensity. Similarly, P60 amplitude increased with increasing TMS intensity; however, the latency tended to

Table 1. Mean amplitude and latency of TMS-evoked potentials following single pulse TMS at different intensities

	TMS Intensity (%RMT)			P Value
	110	125	140	
P30				
Amplitude, μV	7.2 (6)	9.5 (5)	14.0 (7)	0.05
Latency, ms	30.7 (3)	33.9 (4)*	34 (2)*†	0.001
P60				
Amplitude, μV	3.9 (4)	9.2 (5)*	18.1 (8)*†	<0.001
Latency, ms	58.2 (5)	54.5 (7)	60.2 (5)	0.02
N100				
Amplitude, μV	–10.1 (7)	–12.1 (9)	–14.0 (7)	0.15
Latency, ms	107.9 (22)	111.5 (14)	121.9 (14)	0.15

Values are means \pm SD. TMS, transcranial magnetic stimulation; RMT, resting motor threshold. P value represents results from one-way repeated measures ANOVA. * $P < 0.05$, compared with 110% RMT. † $P < 0.05$, compared with 125% RMT.

be shorter at 125% RMT. N100 amplitude and peak latency also tended to increase with increasing TMS intensity; however, this failed to reach significance.

Altering conditioning intensities. Following paired-pulse TMS, test MEPs at S1mV were significantly inhibited following conditioning intensities of 120% ($P = 0.018$) and 140% RMT ($P = 0.009$) but not 100% RMT ($P = 0.373$). Consistent with this, LIC_{MEP} strength increased with increasing conditioning intensities and was greatest at 140% ($P = 0.014$, fig. 1E).

Figure 1, A–C, describes group averages of test TEP waveforms following single-pulse TMS at S1mV and paired-pulse TMS with conditioning intensities of 100, 120, and 140% RMT. Test TEPs at P30 were significantly inhibited following conditioning intensities at 120% ($P = 0.024$) and 140% RMT ($P = 0.003$), but not 100% RMT ($P = 0.99$). Concurrently, LIC_{P30} strength increased with increasing conditioning intensities and was greatest at 140% RMT ($P = 0.001$). Test TEPs at P60 were also inhibited following conditioning intensities at 120% ($P = 0.049$) and 140% RMT ($P = 0.009$) but not 100% RMT ($P = 0.759$). LIC_{P60} strength also increased with increasing conditioning intensities ($P = 0.009$). In contrast, N100 was significantly inhibited at conditioning intensities at 100% RMT ($P = 0.027$) and tended to be inhibited at conditioning intensities of 120% RMT ($P = 0.12$) but not 140% RMT ($P = 0.20$). Overall, increasing conditioning intensity had no significant effect on LIC_{N100} strength ($P = 0.42$; Fig. 1F).

The negative N100 slope resulting from the conditioning pulse (–30 to –5 ms before the test pulse, see Fig. 1, A–C) became significantly more negative with increasing conditioning intensities ($P = 0.002$; Fig. 2A). The steepness of this slope negatively correlated with LIC_{MEP} ($P = 0.016$; Fig. 2B) and LIC_{P30} ($P < 0.001$; Fig. 2C) across all conditioning intensities. There was no relationship between conditioning N100 slope and LIC_{P60} ($P = 0.31$) or LIC_{N100} ($P = 0.62$).

Altering test intensities. Following a constant conditioning intensity at S1mV, test MEPs at 110% ($P = 0.012$), 125% ($P = 0.003$), and 140% RMT ($P = 0.048$) were significantly inhibited. LIC_{MEP} decreased with increasing test intensities and was significantly reduced at 140% RMT ($P = 0.03$; Fig. 3E).

Figure 3, A–C, describes group averages of test TEP waveforms following single-pulse TMS at 110, 125, and 140% RMT and paired-pulse TMS at conditioning intensities of S1mV and test intensities corresponding to single-pulse trials. P30 was significantly inhibited at test intensities of 110% RMT ($P = 0.024$) and tended to be inhibited at test intensities of 125% ($P = 0.054$) and 140% RMT ($P = 0.054$). Increasing test intensities decreased LIC_{P30} strength, which was significantly decreased at test intensities of 140% RMT ($P = 0.002$). P60 tended to be inhibited at test intensities of 110% RMT ($P = 0.072$) and was not inhibited at test intensities of 125% ($P = 0.303$) and 140% RMT ($P = 0.99$). Increasing test intensities decreased LIC_{P60} strength ($P = 0.012$). With constant conditioning intensity at S1mV, N100 was not inhibited at any test intensity ($P > 0.3$). Increasing test intensity strength also had no significant effect on LIC_{N100} strength ($P = 0.78$; Fig. 3F).

As was expected, there was no change in the conditioning N100 slope with constant conditioning intensities ($P = 0.4$). Steepness of conditioning N100 slope did not correlate with LIC_{MEP} ($P = 0.62$), LIC_{P30} ($P = 0.09$), LIC_{P60} ($P = 0.196$), or LIC_{N100} ($P = 0.08$) across different test intensities.

Correlations. When inhibition data were compared across all conditions (both altered conditioning and test intensity), there was a significant positive relationship between LIC_{P30} and LIC_{MEP} ($P = 0.001$; Fig. 4A). LIC_{P30} also positively correlated with LIC_{P60} ($P = 0.033$; Fig. 4B), although there was no correlation between LIC_{P60} and LIC_{MEP} ($P = 0.355$) or LIC_{N100} ($P = 0.1$). There was no significant correlation between LIC_{N100} strength and either LIC_{P30} ($P = 0.52$) or LIC_{MEP} ($P = 0.44$) when compared across conditions. There were no significant correlations among single-pulse MEP, P30, or N100 amplitudes (all $P > 0.3$). P60 amplitude was positively correlated with MEP amplitude ($P = 0.039$) and negatively correlated with N100 amplitude ($P = 0.016$).

DISCUSSION

This study has three main findings. First, altering both conditioning and test intensities modulated LICI strength of the MEP (LIC_{MEP}) and early TEP components (LIC_{P30} and

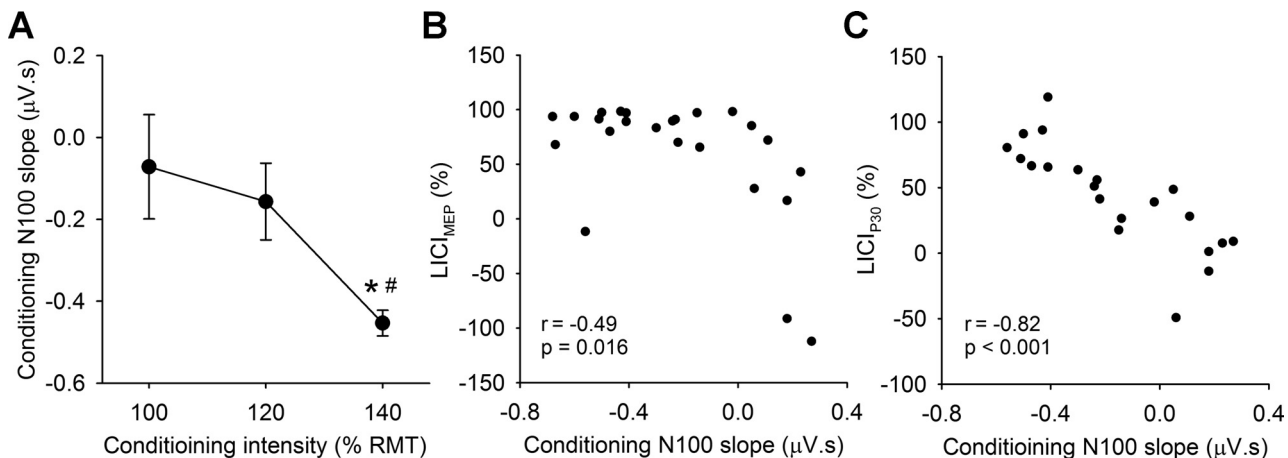


Fig. 2. A: changes in N100 slope (mean derivative) evoked by the conditioning pulse at different conditioning intensities. B: Spearman's rank correlation between long-interval cortical inhibition strength measured from motor-evoked potentials (LIC_{MEP}) and conditioning N100 slope across all conditioning intensities. C: Spearman's rank correlation between LICI strength measured from TMS-evoked potential component P30 (LIC_{P30}) and conditioning N100 slope across all conditioning intensities. Note that raw data are presented, not ranked data used for correlations. * $P < 0.05$ compared with 100% RMT. # $P < 0.05$ compared with 120% RMT.

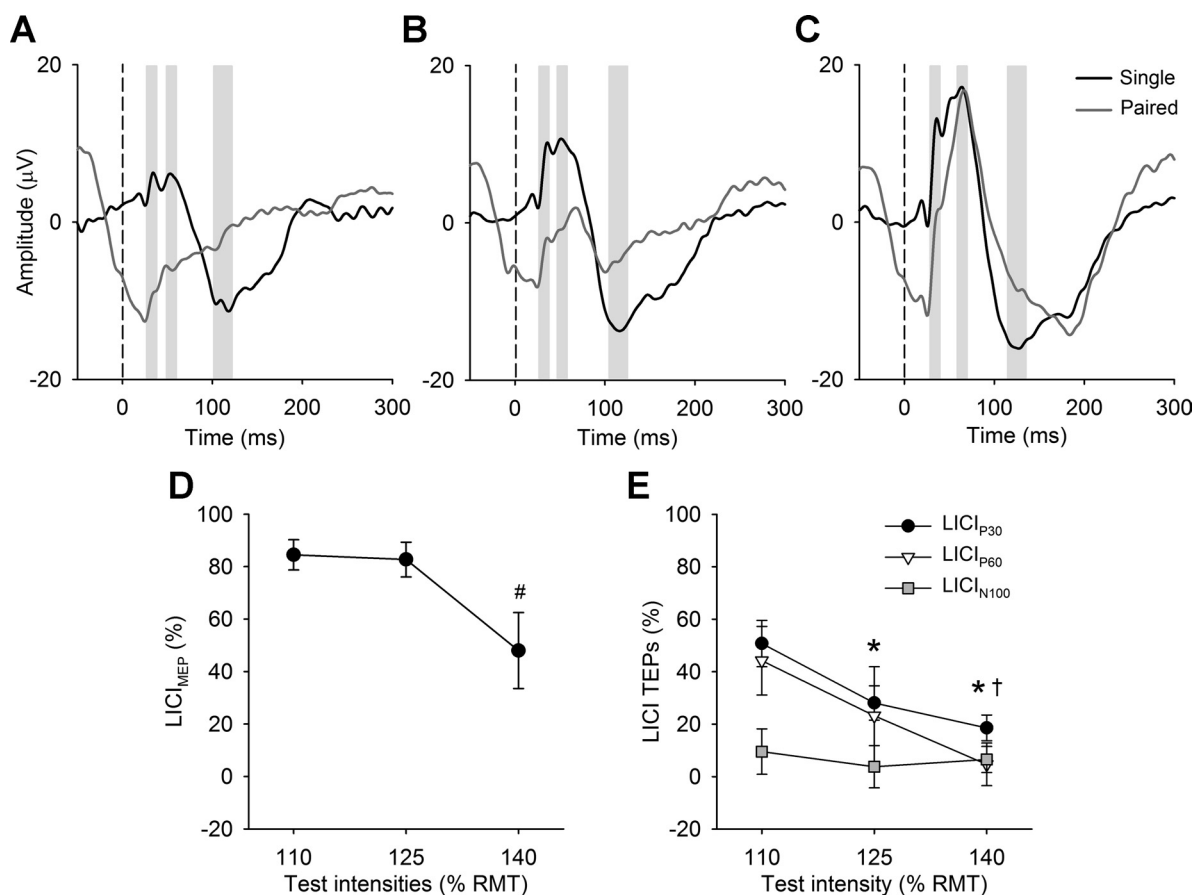


Fig. 3. A–C: group averaged TEPs over motor cortex following single (black) and paired (grey) pulses. Conditioning intensities were constant at 1 mV whereas test intensities for single and paired pulses were 110% (A), 125% (B), and 140% (C) of RMT. Dashed lines marks the test pulse. Gray boxes mark the average time window used for long-interval cortical inhibition measures (LICI_{P30}, left; LICI_{P60}, middle; and LICI_{N100}, right). D and E: mean LICI strength measured at a constant conditioning intensity (1 mV) and different test intensities (110, 125, and 140% RMT). LICI was measured from motor-evoked potentials (LICI_{MEP}; D) and TEP components (LICI_{P30}, LICI_{P60}, and LICI_{N100}; E). *LICI_{P30}, $P < 0.05$ compared with 110% RMT. #LICI_{MEP}, $P < 0.05$ compared with 125% RMT. †LICI_{P60}, $P < 0.05$ compared with 110% RMT.

LICI_{P60}), but not a later component (LICI_{N100}). The strength of LICI_{MEP}, LICI_{P30}, and LICI_{P60} increased with increasing conditioning intensities and decreased with increasing test intensities. LICI_{N100} remained unchanged across both conditions. Second, LICI_{P30} significantly correlated with LICI_{MEP} when compared across all conditions. Third, both LICI_{P30} strength and LICI_{MEP} strength were related to the slope of the N100 following the conditioning TMS pulse, whereas LICI_{P60} and LICI_{N100} were not. These findings provide greater insights into the cortical mechanisms underlying both MEP and TEP inhibition using the LICI paradigm. Our findings also provide additional supporting evidence for the cortical origins of different TEP components.

Mechanism underlying LICI. The primary candidate mechanism underlying LICI is slow GABA_B-mediated inhibition resulting from the conditioning TMS pulse (McDonnell et al. 2006; Nakamura et al. 1997). The excitability of the cortex plays an important role in determining the cortical response to TMS (Silvanto et al. 2008), and fluctuations in cortical excitability are reflected in the EEG (Buzsaki et al. 2012). For instance, slow oscillatory up and down states influence MEP and TEP amplitude during the early stages of sleep (Bergmann et al. 2012) and both alpha (8–12 Hz; Sauseng et al. 2009; Zarkowski et al. 2006) and mid-range beta (15–18 Hz; Maki and Ilmoniemi 2010a) oscillations immediately preceding the

TMS pulse are associated with MEP amplitude in awake participants. In the current study, increasing conditioning pulse intensity increased the slope of the N100 following the conditioning pulse, the peak of which coincided with delivery of the test TMS pulse. Increased conditioning N100 slope was accompanied by greater LICI_{MEP} and LICI_{P30} following the test TMS pulse, suggesting an inhibitory role of N100. The increase in conditioning N100 slope and inhibition with increasing TMS intensities is consistent with the higher threshold required for postsynaptic GABA_B-related inhibition (Connors et al. 1988; Dutar and Nicoll 1988) and replicates previous LICI_{MEP} studies (Hammond and Garvey 2006; Valls-Sole et al. 1992). In contrast to altered conditioning intensity, the conditioning N100 slope remained constant with constant conditioning intensities and both LICI_{MEP} and LICI_{P30} strength decreased with increasing test TMS intensities. This evidence replicates findings from previous LICI_{MEP} studies (Daskalakis et al. 2002; Sanger et al. 2001) and is also consistent with brain slice studies suggesting that postsynaptic GABA_B-mediated inhibition can be overcome with strong excitatory inputs (Connors et al. 1988). Alternatively, reduced LICI at higher TMS intensities may indicate that lower threshold neurons are more sensitive to LICI than higher threshold neurons (Sanger et al. 2001). Regardless, with constant test intensities LICI of both

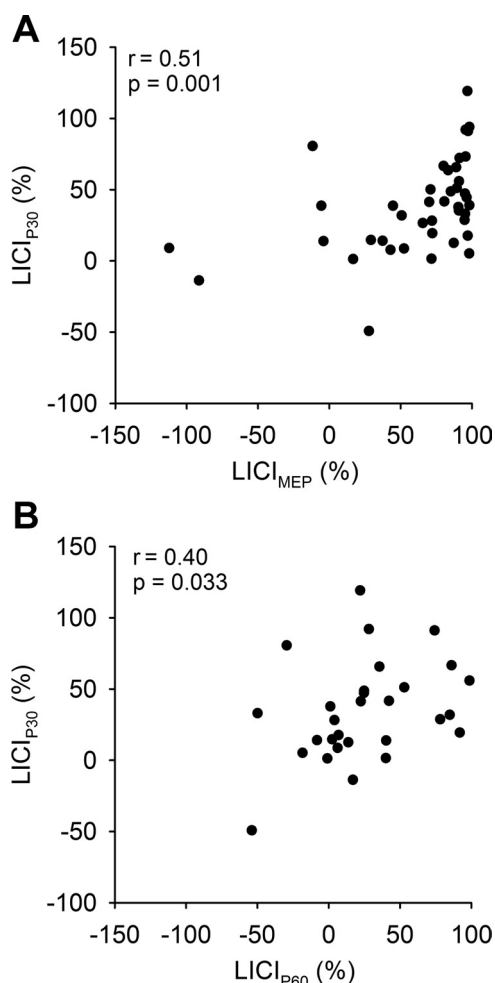


Fig. 4. Pearson's correlation between measures of LICI. A: motor-evoked potentials ($LICI_{MEP}$) compared with TMS-evoked potential component P30 ($LICI_{P30}$) across all conditions. B: TEP component P30 ($LICI_{P30}$) compared with TMS-evoked potential component P60 ($LICI_{P60}$) across all conditions.

MEPs and TEPs appear related to the N100 evoked by the conditioning pulse.

Several lines of evidence from previous studies support that N100 reflects GABA_B-mediated inhibition. First, the N100 peak latency is similar to GABA_B-mediated inhibitory post-synaptic potentials following electrical stimulation of cortical tissue in vitro (Bikmullina et al. 2009; Komssi et al. 2004; Nikulin et al. 2003; see Table 1). Second, functional tasks associated with increased cortical inhibition, such as preparing to inhibit the response to TMS (Bonnard et al. 2009) or resisting a perturbation of the wrist (Spieser et al. 2010), are accompanied by increased N100 amplitude. Conversely, tasks associated with decreased inhibition, such as movement preparation, result in reduced N100 amplitude (Bender et al. 2005; Kicic et al. 2008; Nikulin et al. 2003). Third, children with attention deficit hyperactivity disorder, a condition associated with reduced cortical inhibition, have reduced N100 amplitudes compared with healthy children (Bruckmann et al. 2012). Paradoxically, several studies have found opposing modulation of the N100 during periods of increased cortical inhibition. Acute alcohol consumption, a state associated with increased cortical inhibition, results in decreased N100 amplitude (Kahkonen and Wilenius 2007). Similarly, stimulation of peripheral

afferents results in a period of increased cortical inhibition deemed short-latency afferent inhibition; however, N100 amplitude during short-latency afferent inhibition is also reduced (Bikmullina et al. 2009; Ferreri et al. 2012). The finding from the current study that increased conditioning N100 slope relates to increased $LICI_{MEP}$ and $LICI_{P30}$ supports an inhibitory origin of the N100, most likely via a GABA_B inhibitory mechanism. Importantly, the N100 evoked by the conditioning pulse appears to reflect the mechanism underlying $LICI_{MEP}$ and $LICI_{P30}$ in the motor cortex.

Origins of MEP and TEP inhibition. MEPs and TEPs are complimentary but independent measures of cortical excitability (Huber et al. 2012). MEP amplitude is influenced by cortical and spinal excitability as well as changes in muscle properties. $LICI_{MEP}$ is thought to originate in the cortex as corticospinal descending volleys are also decreased with LICI, suggesting decreased cortical output (Di Lazzaro et al. 2002; Nakamura et al. 1997). In addition, the responses to paired transcranial electrical stimuli are not altered following a conditioning TMS pulse, suggesting no alterations in spinal excitability with $LICI_{MEP}$ (Inghilleri et al. 1993). However, a recent study using paired stimulation of the cervicomedullary junction at ISIs of 100 ms revealed a similar inhibitory pattern to stimulation of the cortex with TMS, suggesting a spinal contribution to $LICI_{MEP}$ is possible (McNeil et al. 2011). The relationship between the conditioning N100 slope and $LICI_{MEP}$ in the current study supports a cortical origin for $LICI_{MEP}$. Although a spinal contribution cannot be eliminated, the current data suggest at least 50% of $LICI_{MEP}$ is related to cortical inhibitory mechanisms.

Compared with MEPs, TEPs represent fluctuations in excitation and inhibition of local cortical populations and wider cortico-cortical and cortico-thalamic networks (Ilmoniemi and Kicic 2010; Rogasch and Fitzgerald 2012; Siebner et al. 2009). Several authors have suggested that different temporal components of the TEP may reflect independent cortical mechanisms (Komssi et al. 2004; Nikulin et al. 2003). In support of this notion, we found differential modulation of $LICI_{P30}/LICI_{P60}$ and $LICI_{N100}$ with altered stimulation parameters. Several findings suggest that early TMS-evoked potentials such as P30 are related to excitatory activity resulting from TMS. Paired-pulse TMS studies have shown that test MEPs (Valls-Sole et al. 1992) and descending corticospinal volleys (Nakamura et al. 1997) are facilitated at ISIs of 20–50 ms following a suprathreshold conditioning pulses. A facilitated response suggests a period of increased cortical excitation around the latency of P30. In TMS-EEG studies, early peaks around P30 increase in a sigmoidal fashion with increased TMS intensity, similar to MEP amplitude (Komssi et al. 2004). In addition, peak-to-peak amplitude of N15/P30 TEP is related to MEP amplitude (Maki and Ilmoniemi 2010b) and N15, P30, and MEP amplitude are diminished by positioning the TMS coil at nonoptimal angles (Bonato et al. 2006). With the use of paired-pulse paradigms to assess SICI and intracortical facilitation, contralateral P30 modulation is comparable to MEP modulation (Ferreri et al. 2011), although this pattern has not been consistently found (Paus et al. 2001). We observed a positive relationship between $LICI_{MEP}$ and $LICI_{P30}$ across a range of conditioning and test intensities. Given that reduced MEP amplitude is most likely dependent on decreased output

from corticospinal neurons, $LICI_{P30}$ is consistent with suppression of excitatory mechanisms in the cortex.

The origin of the P60 component is less clear. P60 may partially reflect reafferent joint or cutaneous sensory potentials resulting from TMS-evoked finger movement (Paus et al. 2001). In support of this we found a positive correlation between MEP amplitude and P60 amplitude following single-pulse TMS. P60 amplitude was also negatively correlated with N100 amplitude suggesting a possible association with inhibitory mechanisms. In contrast, $LICI_{P60}$ was modulated in a similar pattern to $LICI_{MEP}$ and correlated with $LICI_{P30}$ following alterations in LICI stimulus parameters. This inhibitory pattern of P60 is consistent with suppression of excitatory mechanisms. Further investigation into the cortical origin of P60 controlling for reafferent feedback is needed, such as during deafferentation of the arm with ischaemic nerve block.

As established earlier, the N100 component of the TMS-evoked potential is likely to reflect $GABA_B$ -mediated inhibition. However, the mechanism underlying $LICI_{N100}$ following the test pulse remains unclear. If the N100 is inhibitory, a decrease in test N100 amplitude is therefore suggestive of decreased inhibitory input to pyramidal cells from inhibitory interneurons (Pell et al. 2011; Rogasch and Fitzgerald 2012). In rat and human brain slice studies, paired-pulse depression of $GABA_B$ -mediated inhibitory postsynaptic potentials in pyramidal cells results from suppression of GABA release by presynaptic autoinhibition (Davies et al. 1990; Deisz 1999; Deisz and Prince 1989). Autoinhibition results from $GABA_B$ "autoreceptors" located on the presynaptic terminal of inhibitory interneurons (Davies et al. 1990; Deisz and Prince 1989). In humans, this phenomenon has been demonstrated in TMS studies by assessing the effect of LICI on other inhibitory networks such as those that mediate SICI (Cash et al. 2010; Sanger et al. 2001). $LICI_{100}$ may directly represent $GABA_B$ -mediated presynaptic inhibition of inhibitory neurotransmission similar to paired-pulse depression in slice studies. There are several possibilities that could explain the lack of $LICI_{N100}$ modulation observed in the current study. As presynaptic inhibition of interneurons requires minimal GABA release (Kobayashi et al. 2012), the lack of $LICI_{N100}$ modulation may reflect saturated presynaptic inhibition of inhibitory interneurons. Alternatively, the low levels of $LICI_{N100}$ observed (~20% across conditions with inconsistent significance) may have limited the sensitivity of TMS-EEG to detect changes in inhibition strength. Finally, the slow recovery of the N100 evoked by the conditioning pulse may have masked inhibition of the N100 evoked by the test pulse, particularly at high conditioning intensities. This is evident in Figs. 1C and 3A where no clear N100 waveform is present following the conditioned test pulse despite minimal $LICI_{100}$ measured using the current technique. Future studies accounting for the N100 evoked by the conditioning pulse would be beneficial in clarifying this potential effect.

Limitations. There are several important limitations to the current study. Although we have attributed the TEP components and $LICI_{P30}$ and $LICI_{N100}$ to local cortical networks, other sources are possible, such as distant cortical or subcortical regions (Ilmoniemi et al. 1997; Litvak et al. 2007). For instance, source localization studies have found both ipsilateral and contralateral sources contribute to the P30 component (Komssi et al. 2002; Litvak et al. 2007) and contralateral P30

is reduced using the SICI paradigm (Ferrerri et al. 2011). Unfortunately, the limited electrode montage used in the current study did not extend to the contralateral hemisphere precluding measurements of inhibition at such sites. Recordings using larger electrode arrays would allow better spatial characterization of $LICI_{P30}$, $LICI_{P60}$, and $LICI_{N100}$. Discharge of the TMS coil results in a loud clicking noise that can induce auditory evoked potentials (Tiitinen et al. 1999). These evoked potentials have a similar time course to the N100 component and are both air and bone conducted (Nikouline et al. 1999). We masked the air conducted component by playing white noise through ear phones (Massimini et al. 2005). Comparisons between high-intensity sham stimulation and low-intensity real stimulation confirmed that this approach was sufficient to minimize auditory evoked potentials in our recordings. Somatosensory evoked potentials resulting from muscle and joint movement in the hand muscle following TMS may contaminate TEP components (Paus et al. 2001; Schürmann et al. 2001). However, such potentials would occur at least 40 ms after TMS allowing for conduction to and from the periphery and therefore cannot explain the relationship between $LICI_{MEP}$ and $LICI_{P30}$. We found no correlations between $LICI_{MEP}$ and $LICI_{N100}$, suggesting this component is also not related to somatosensory evoked potentials. In addition, LICI of TEPs has been shown in nonmotor brain regions in previous studies, removing this potential confound (Daskalakis et al. 2008; Fitzgerald et al. 2008). Indeed, replication of these results in other brain regions would strengthen the conclusions regarding the origin of different TEP components. TMS can also stimulate trigeminal nerve afferents either by direct depolarization or via small coil vibrations on the scalp. Stimulation of trigeminal nerves over the supraorbital foramen results in bilateral inhibition of motor cortical areas between 30 and 65 ms (Siebner et al. 1999). However, this time course of inhibition is shorter than that reported here (ISI = 100 ms). Sham designs incorporating electrical stimulation of the scalp will further assist in controlling for this potential source of contamination. Finally, the sample size used in the current study is small. The number of participants included was based on previous TMS studies addressing similar inhibitory phenomena (Sanger et al. 2001; Valls-Sole et al. 1992). Although the sample size is small, this number appears adequate to demonstrate the relatively robust inhibitory effects of paired-pulse TMS both in EMG and EEG. Regardless, replication in a larger sample is required.

Conclusions. We have demonstrated that LICI of early ($LICI_{P30}$ and $LICI_{P60}$) and late ($LICI_{N100}$) TEP components are differentially modulated with altered stimulation parameters. $LICI_{P30}$ is consistent with suppression of excitatory mechanisms, whereas $LICI_{N100}$ may reflect presynaptic autoinhibition of inhibitory neurotransmission. Finally, the N100 evoked by the conditioning pulse is consistent with the underlying mechanism resulting in $LICI_{MEP}$ and $LICI_{P30}$, most likely postsynaptic $GABA_B$ -mediated inhibition. Inhibition of different TEP components may be used to assess the role of pre- and postsynaptic inhibitory mechanisms in brain function and dysfunction, such as in psychiatric conditions. Confirmation of this finding over other brain regions and with a larger sample is required.

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AUTHOR CONTRIBUTIONS

Author contributions: N.C.R., Z.J.D., and P.B.F. conception and design of research; N.C.R. performed experiments; N.C.R. analyzed data; N.C.R., Z.J.D., and P.B.F. interpreted results of experiments; N.C.R. prepared figures; N.C.R., Z.J.D., and P.B.F. drafted manuscript; N.C.R., Z.J.D., and P.B.F. edited and revised manuscript; N.C.R., Z.J.D., and P.B.F. approved final version of manuscript.

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