

# Augmenting Plasticity Induction in Human Motor Cortex by Disinhibition Stimulation

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**Cellular studies showed that disinhibition, evoked pharmacologically or by a suitably timed priming stimulus, can augment long-term plasticity (LTP) induction. We demonstrated previously that transcranial magnetic stimulation evokes a period of presumably GABA<sub>B</sub>ergic late cortical disinhibition (LCD) in human primary motor cortex (M1). Here, we hypothesized that, in keeping with cellular studies, LCD can augment LTP-like plasticity in humans. In Experiment 1, patterned repetitive TMS was applied to left M1, consisting of 6 trains (intertrain interval, 8 s) of 4 doublets (interpulse interval equal to individual peak I-wave facilitation, 1.3–1.5 ms) spaced by the individual peak LCD (interdoublet interval (IDI), 200–250 ms). This intervention (total of 48 pulses applied over ~45 s) increased motor-evoked potential amplitude, a marker of corticospinal excitability, in a right hand muscle by 147% ± 4%. Control experiments showed that IDIs shorter or longer than LCD did not result in LTP-like plasticity. Experiment 2 indicated topographic specificity to the M1 hand region stimulated by TMS and duration of the LTP-like plasticity of 60 min. In conclusion, GABA<sub>B</sub>ergic LCD offers a powerful new approach for augmenting LTP-like plasticity induction in human cortex. We refer to this protocol as disinhibition stimulation (DIS).**

**Keywords:** I-waves, late cortical disinhibition, LTP-like plasticity, motor cortex, transcranial magnetic stimulation

## Introduction

There are a number of *in vitro* stimulation protocols that can induce long-term plasticity (LTP) of excitatory synapses across a variety of experimental preparations (Thickbroom 2007). However, *in vivo* excitatory synapses are typically components of more complex networks that include excitatory and inhibitory connections, and there is evidence that the level of inhibitory tone in the neuronal network can modulate the efficacy of LTP. Theta-burst stimulation of superficial layers of rat primary motor cortex (M1) typically fails to induce LTP unless there is simultaneous blockade of intracortical inhibition either by a pharmacological agent such as the GABA<sub>A</sub> receptor antagonist bicuculline (Wigstrom and Gustafsson 1983a, 1983b, 1985; Hess and Donoghue 1994; Castro-Alamancos et al. 1995; Hess et al. 1996; Hess and Donoghue 1996) or by tetanization of vertical afferents, which reduce the efficacy of inhibitory transmission (Iriki et al. 1989; Hess et al. 1996). Likewise, induction of associative LTP fails in piriform cortex unless GABA<sub>A</sub> receptor activity is blocked (Kanter and Haberly 1993).

Network considerations are also relevant to human studies of LTP-like plasticity that employ noninvasive brain stimulation to M1, and in keeping with experimental observations, there is evidence that the level of cortical inhibition can be important

in the human also. The induction of an LTP-like increase in corticospinal excitability with paired-associative stimulation is reduced by diazepam and tiagabine, which increase neurotransmission through the GABA<sub>A</sub> receptor (Heidegger et al. 2010) and by the GABA<sub>B</sub> receptor agonist baclofen (McDonnell et al. 2007). In contrast, ischemic nerve block, which decreases GABA-related cortical inhibition (i.e., disinhibition, Levy et al. 2002), enhances the LTP-like effects of repetitive transcranial magnetic stimulation (rTMS) and practice-dependent plasticity in healthy participants (Ziemann, Corwell et al. 1998; Ziemann, Hallett et al. 1998; Ziemann et al. 2001) and in people who have suffered a stroke (Muellbacher et al. 2002). Pretreatment with lorazepam, a positive allosteric modulator of the GABA<sub>A</sub> receptor, reduces these effects (Ziemann, Hallett et al. 1998; Bütefisch et al. 2000; Ziemann et al. 2001; Teo et al. 2009).

While the level of inhibition can be manipulated pharmacologically, disinhibition can also be evoked electrophysiologically by the application of a suitably timed priming stimulus (PS). In cellular preparations, a PS to an inhibitory neuron leads to a reduction in the IPSP evoked by a test stimulus given a few hundred milliseconds later (paired-pulse depression; Diamond et al. 1988; Davies et al. 1990; Davies et al. 1991; Mott and Lewis 1991; Nathan and Lambert 1991; Otis et al. 1993; Deisz et al. 1997; Deisz 1999). The PS is thought to activate pre- and post-synaptic GABA<sub>B</sub> receptors with different effects: Postsynaptic activation brings about inhibition, whereas presynaptic activation inhibits further GABA release and results in disinhibition. With paired-pulse depression, the time-course of presynaptic disinhibition outlasts that of postsynaptic inhibition resulting in a late period during which disinhibition dominates (Otis et al. 1993; Deisz 1999). Postsynaptic depolarization (Larson and Lynch 1986; Pacelli et al. 1989) and NMDA receptor activation (Davies and Collingridge 1996) at excitatory synapses are facilitated during this period of disinhibition, and these effects have been harnessed to accelerate the induction of synaptic plasticity (Larson and Lynch 1986; Larson et al. 1986; Rose and Dunwiddie 1986; Staubli and Lynch 1987; Diamond et al. 1988; Greenstein et al. 1988; Pacelli et al. 1989; Davies et al. 1991; Mott and Lewis 1991).

A similar period of late cortical disinhibition (LCD) has recently been described in human M1 (Cash et al. 2010, 2011). If a PS is delivered by TMS, it evokes release of GABA from inhibitory interneurons that activates postsynaptic GABA<sub>A</sub> receptors leading to short-lasting (<20 ms) postsynaptic inhibition in the form of short-interval intracortical inhibition (SICI) (Kujirai et al. 1993; Ziemann et al. 1996; Hanajima et al. 1998; Di Lazzaro et al. 2000, 2006), and GABA<sub>B</sub> receptors leading to

long-lasting (100–200 ms) postsynaptic inhibition in the form of long-interval intracortical inhibition (LICI) (Valls-Sole et al. 1992; McDonnell et al. 2006; Müller-Dahlhaus et al. 2008). Disinhibition occurs during the LICI phase (Werhahn et al. 1999; Sanger et al. 2001; McDonnell et al. 2006; Chu et al. 2008; Ni et al. 2011) but has been shown to outlast LICI and result in a period in which disinhibition dominates (LCD, Cash et al. 2010). Disinhibition is demonstrated by the reduction in SICF that occurs around 100–250 ms following PS, and LCD is the period of disinhibition that continues beyond LICI (i.e., from ~190 to 250 ms). As with cellular studies, LCD is associated with increased net excitability, as reflected by increased motor-evoked potential (MEP) amplitude evoked by a single test-pulse delivered between 190 and 250 ms following PS (long-interval cortical facilitation; LICF). Short-interval intracortical facilitation (SICF) is likewise increased during LCD, and indicative of increased excitability at the cortical level (Cash et al. 2008, 2010, 2011). In summary, although this has not been directly proven, for example, by pharmacological studies, LCD most likely reflects presynaptic GABA<sub>B</sub>ergic disinhibition (cf. Supplementary Figure), similar to paired-pulse depression at the cellular level.

We postulated that LCD can be harnessed to improve the efficacy of LTP-like plasticity induction in human cortex. In the present study, we have tested this by combining LCD and a plasticity-inducing protocol known as I-wave TMS (ITMS; Thickbroom et al. 2006). ITMS targets facilitatory I-wave networks and increases excitability by repeatedly delivering paired-pulses at an interpulse interval (IPI) corresponding to the periodicity of I-waves (~1.5 ms) (Cash et al. 2009, 2013).

In the present study, a series of trains consisting of 4 ITMS doublets was delivered such that each doublet in the train (after the first) was delivered during the LCD evoked by its preceding doublet. We show that this intervention is topographically specific and highly effective in inducing LTP-like plasticity when the doublets are presented at LCD periodicity, but not at intervals shorter or longer than LCD. We refer to this protocol as disinhibition stimulation (DIS).

## Methods

### Participants

In Experiment 1, 11 healthy volunteers were screened for the presence of LICF and SICF. Of these, 8 [3 female, aged 22–35 years; right hand dominant according to the Edinburgh Inventory (Oldfield 1971)], were recruited to participate in the study. A further 11 healthy volunteers were screened in the same manner for Experiment 2 (see also Section Experiment 2: duration of effects and topographic specificity) to investigate the duration of effects and topographic specificity, 9 of whom were then recruited (4 female, ages 22–44). All participants gave informed written consent and completed a safety questionnaire prior to the study, which had the approval of the institution's ethics board (Goethe-University of Frankfurt am Main or University Health Network, Toronto) and conformed to the Declaration of Helsinki. Participants were seated comfortably and were asked to remain at rest but alert with eyes open for the duration of the study.

### Electromyography

MEPs were recorded using surface electrodes placed in a belly-tendon arrangement over the first dorsal interosseous (FDI) target muscle of the right hand. In Experiment 2, surface electromyography (EMG) was additionally recorded from abductor pollicis brevis (APB), extensor carpi radialis (ECR), and biceps brachii (BB) muscles.

The peak-to-peak amplitude of the FDI MEP was the primary outcome measure. The electromyographic signal was amplified and filtered (20 Hz–2 kHz), digitized (sample rate 4 kHz) using a CED Micro 1401 (Cambridge Electronic Design), monitored, and stored on a computer.

### TMS: Setup Measurements

Stimulus parameters were investigated and optimized for each individual. TMS was delivered using 3 linked magnetic stimulators (Magstim) with a monophasic current waveform connected to a 70 mm figure-of-eight coil placed over the optimal scalp position (determined from initial exploration) for the hand area of the left M1, and orientated 45° to the sagittal plane so that the induced current in the brain was directed from posterior to anterior (PA). This coil orientation is considered optimal for activating corticospinal neurons trans-synaptically (Di Lazzaro et al. 2008). Resting motor threshold (RMT) at the optimal scalp position was tested by the relative frequency method (Groppa et al. 2012) and defined as the lowest stimulator intensity eliciting MEPs from the right FDI of >50  $\mu$ V peak-to-peak amplitude in at least 5 of 10 trials. The test stimulus intensity that generated a MEP of 1 mV ( $SI_{1mV}$ ) was also determined.

### Determining IPI for Maximum SICF

Paired-pulse TMS (each pulse 110% RMT) were delivered at IPIs in the range 1.1–2.3 ms in 0.2 ms steps. All IPIs, together with single-pulse TMS (at 110% RMT) were delivered in blocks of 8 stimuli (blocks in a pseudo-random order). Averaging peak-to-peak FDI MEP amplitude for each IPI, and expressing this as a percentage of the mean test MEP amplitude, generated a SICF curve. For the remainder of the study, ITMS doublets were delivered at the IPI for which each participant's SICF curve was maximal.

### Determining Interdoublet Interval for Maximum LICF

This measurement was undertaken to confirm that LICF was present after an ITMS doublet, as it is after single-pulse (SP) TMS (Cash et al. 2010, 2011), and to optimize the IDI for maximum LICF. A triple pulse was delivered consisting of an ITMS doublet (110% RMT) followed by a test stimulus (TS, SP TMS,  $SI_{1mV}$ ) at intervals in the range 150–300 ms (150, 190, 200, 210, 220, 250 and 300 ms; blocks of 10 triplets at each interval delivered in pseudo-randomized order, see Fig. 2b insert). The mean TS MEP amplitude at each interval was expressed as a percentage of mean TS MEP alone to generate a LICF curve. The optimal LICF interval (OPT) was determined from the maximum of this curve for each individual and was used as the IDI for the primary intervention.

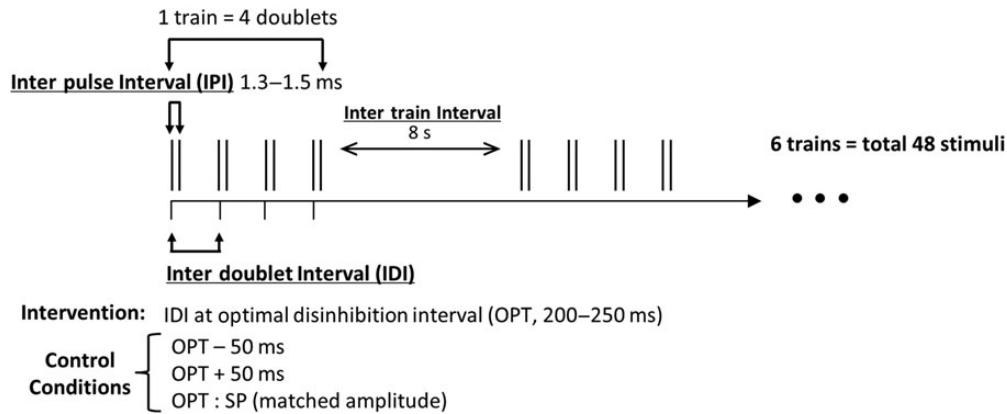
### Influence of ITMS Doublet Intensity on LICF

The intensity of ITMS doublets (each pulse in the doublet was always of equal intensity) was varied (pseudo-randomly, blocks of 10 doublets per intensity) between 90% and 120% RMT in 10% steps. Single-pulse TS of  $SI_{1mV}$  were delivered at OPT after each doublet (see Fig. 2c insert), and the doublet intensity that gave maximal LICF was determined for each individual.

### TMS: Intervention Protocol and Corticomotor Excitability

This phase of the study required the use of a MagPro X100 magnetic stimulator (MagVenture) to deliver the necessary pattern and frequency of stimulation. A 75 mm figure-of-eight coil (biphasic pulse, AP-PA-induced current direction in the brain, 0.28 ms duration pulse), connected to the MagPro, was held over the optimal scalp position for activating the right FDI (as determined by initial exploration), and orientated 45° lateral to the midline.

Single-pulse RMT was determined according to the criteria described above. A train of 4 ITMS doublets was then delivered at an IDI given by OPT, and stimulator intensity was adjusted (in the range 100%–120% RMT) to determine the minimum intensity that resulted in an increase in doublet MEP amplitude across the train. For safety reasons, this minimum intensity was used for all subsequent doublet protocols.



**Figure 1.** Intervention protocol: The primary TMS intervention consisted of 6 trains (intertrain interval, 8 s) of 4 ITMS doublets (interpulse interval equal to individual peak I-wave facilitation, 1.3–1.5 ms) spaced by the individual optimal disinhibition interval (OPT, 200–250 ms). A total of 48 pulses were applied over ~45 s. The OPT interval was determined using triple-pulse TMS (see Fig. 2a). Control conditions consisted of decreasing (OPT – 50 ms) or increasing (OPT + 50 ms) the IDI, and replacing ITMS with amplitude-matched SP TMS. SP MEPs were recorded pre- and post-intervention to investigate effects on cortical excitability.

The primary TMS intervention consisted of 6 of these trains delivered at an intertrain interval of 8 s (Fig. 1). The intervention thus consisted of 48 stimuli delivered over ~45 s (6 trains of 4 ITMS doublets, each train 8 s apart).

At baseline, 4 blocks of 10 SP TS (at  $SI_{1mV}$  and  $5 \pm 1.25$  s intervals, ~1-min acquisition time) were delivered, each block 1 min apart, and the mean MEP amplitude of each block was calculated. Postintervention, blocks of 10 TS were delivered at 1, 3, 5, 7, 9, 11, 16, 21, 26, and 31 min, and the mean MEP amplitude for each block calculated.

#### TMS: Control Protocols

Control conditions used the same protocol, except that either the IDI was (1) decreased by 50 ms (OPT – 50;  $n = 8$  participants), (2) increased by 50 ms (OPT + 50;  $n = 8$ ), or (3) IDI was retained at OPT, but doublets were replaced by SP stimuli at an intensity that matched the ITMS doublet MEP amplitude (OPT – SP;  $n = 7$ ).

We used a pseudo-randomized double-blinded crossover design. This was achieved by 2 experimenters: One experimenter performed the intervention, whereas the main experimenter who was blinded toward interventional condition performed all other TMS measurements and was responsible for analyzing the data. Pseudo-randomization followed a preset table of the order of interventional conditions that was fully balanced across participants. This table was sealed away from the main experimenter who was responsible for all TMS measurements and data analysis. In a given subject, experiments were separated by several days but carried out at the same time of day for each subject to control for individual chronotype and diurnal variations in cortisol levels (Sale et al. 2007, 2008) and GABAergic inhibition in motor cortex (Lang et al. 2011), and female participants were asked to avoid bookings that were likely to take place in the first days of the menstrual cycle to minimize potential hormonal confounding effects on neuroplasticity between sessions (Inghilleri et al. 2004). Participants were instructed not to contract hand muscles during or following intervention as this may interact with plastic effects (Gentner et al. 2008; Huang et al. 2008).

#### Experiment 2: Duration of Effects and Topographic Specificity

For this additional experiment, all procedures in Sections Participants; Electromyography; TMS: setup measurements; Determining IPI for maximum SICF; Determining interdoublet interval for maximum LICF were adhered to and the intervention was delivered at optimal intervals as described in Section TMS: intervention protocol and corticomotor excitability. To investigate the topographic specificity of the plasticity protocol, EMG was recorded from FDI as before and additionally from APB, ECR, and BB. To investigate duration of effects, baseline excitability was recorded in the same manner and time-points as described in Section TMS: intervention protocol and corticomotor excitability, and additionally at 45, 60, 90, and 120 min postintervention as well as

14 h later on the following morning. The intervention was administered at approximately 6 PM in all cases.

#### Safety

Participants were instructed to remain alert throughout the interventions and were warned that if they perceived a spread of movement from hand into arm muscles, they should move away from the coil and report this to the investigator (Pascual-Leone et al. 1994). The investigator closely monitored the arm for any indication of a spread of activation. A neurologist was on standby for all intervention experiments. No exact precedent exists for this intervention protocol, and we followed the safety criteria for rTMS guidelines (cf. Fig. 5 in Pascual-Leone et al. 1993; cf. Table 3 in Chen et al. 1997; cf. Table 3 in Wassermann 1998; cf. Table 4 in Rossi et al. 2009) and the minimum intensity of stimulation was chosen that produced an effect across doublets in a train (see above) and was capped at 120% RMT. No participants reported any spread in excitability or moved away from the coil, and there were no adverse effects. Participants were asked for feedback after the studies and none raised concerns about their experience.

#### Data Analysis

##### Effect of IPI

Normalized MEP amplitude versus IPI curves were calculated from mean MEP amplitude (as a percentage of baseline) for each subject, averaged across subjects. A linear mixed model (LMM) with random effect of individual was used to examine the effect of the fixed factor IPI and muscle (where relevant) on normalized MEP amplitude. Restricted maximum likelihood estimation was used in all modeling.

##### Effect of Interventions

To confirm that preintervention baseline MEP amplitude did not differ between interventions, an LMM analysis was performed with intervention as the fixed effect. To determine whether there was an effect of intervention on MEP amplitude over time and a difference across interventions or muscles, LMM analysis was performed with fixed factors “intervention” or “muscle”, and “time” (0 [i.e., preintervention], 1, 3, 5, 7, 9, 11, 16, 21, 26, and 31 min postintervention), and the dependent variable MEP amplitude expressed as a percentage of preintervention baseline. In Experiment 2, additional time-points 45, 60, 90, 120 min, and 14 h were included in the analyses. Random effect of individual was included in all LMM analyses.

Results are expressed as mean  $\pm$  SEM. Datasets for each measure (e.g., SICF, LICF in Section Effect of IPI, and plasticity in Section Effect of interventions) were assessed for normality of data distribution using the Kolmogorov–Smirnov test and log-transformed if necessary to



generate normality as required for valid LMM statistical assessment. When significance of condition main effects was indicated by LMM, post hoc two-tailed *t*-tests (critical *P*-value 0.05) were performed using Fisher's LSD. Correlations were tested using the permutation (exact) test (Ludbrook and Dudley 1998). Statistical analysis was performed using SPSS version 16.0.

## Results

### Experiment 1

#### IPI for Maximum SICF

Group mean RMT was  $52\% \pm 3\%$  of maximum stimulator output. Mean single-pulse MEP amplitude at 110% RMT was  $0.54 \pm 0.09$  mV. The effect of IPI on SICF was significant (LMM:  $F_{6,14} = 3.8$ ;  $P < 0.05$ ). On average, SICF was maximal at an IPI of 1.3 ms, and SICF was significantly greater than SP baseline at IPIs of 1.1, 1.3, and 1.5 ms ( $256\% \pm 37\%$ ,  $339\% \pm 66\%$ , and  $276\% \pm 51\%$  of baseline, respectively;  $P < 0.05$ ; Fig. 2a). Individually, the maximum SICF occurred at an IPI of either 1.3 ms ( $n = 6$  participants) or 1.5 ms ( $n = 2$ ), and subsequently ITMS doublets were delivered at these individually optimal IPIs.

#### IDI for Maximum LICF

There was a significant effect of IDI on LICF (LMM:  $F_{6,8.4} = 4.6$ ,  $P < 0.05$ ). Figure 2b shows that when the TS was delivered after an ITMS doublet, TS MEP amplitude was initially reduced (in keeping with LICF) and then transiently increased (in keeping with LICF)—a pattern similar to that reported after an SP PS (Cash et al. 2010, 2011). On average, the greatest reduction in TS amplitude (i.e., LICF) occurred 150 ms post-ITMS ( $25\% \pm 16\%$  of baseline,  $P < 0.05$ ), and the greatest increase (i.e., LICF) occurred at 220 ms ( $164\% \pm 30\%$  of baseline;  $P < 0.05$ ). The optimal IDI of LICF for each subject occurred at either 220 ms ( $n = 4$ ) or 250 ms ( $n = 4$ ) post-ITMS, and these values

were used to set the IDI for each participant. In all cases, TS amplitude returned to baseline by 300 ms.

#### ITMS Doublet Intensity and LICF

At the optimal IDI, increasing the intensity of the pulses making up the ITMS doublet, from 90% to 120% RMT (in 10% steps), led to a progressive increase in the amplitude of TS delivered at OPT ( $90\% \pm 11\%$ ,  $122\% \pm 13\%$ ,  $175\% \pm 31\%$ , and  $263\% \pm 51\%$ , respectively, of TS baseline; LMM:  $F_{3,8.5} = 5.47$ ,  $P < 0.05$ ). Post hoc *t*-tests revealed that the minimum intensity for a significant increase in TS amplitude (LICF) was 110% RMT (Fig. 2c).

#### Determination of Stimulus Intensity for Intervention

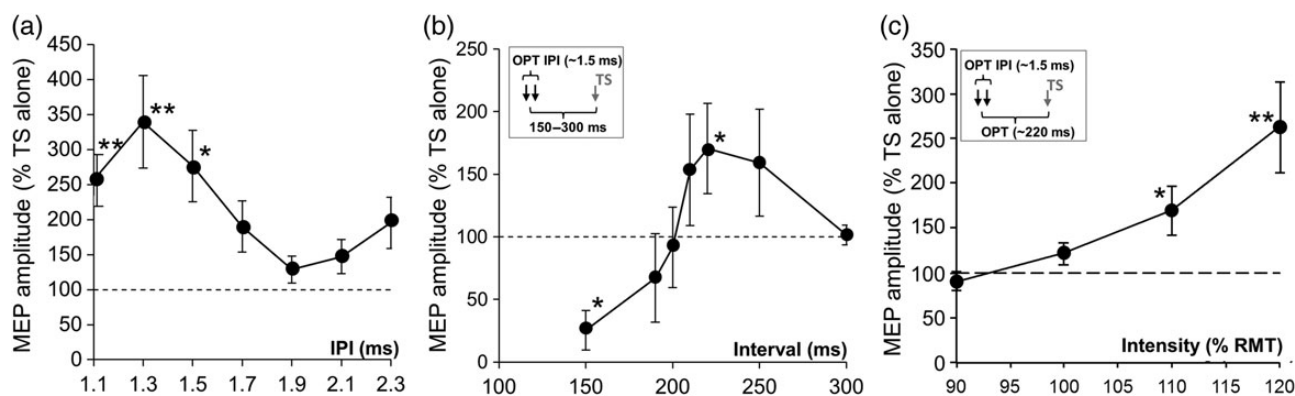
The MEP amplitude of ITMS doublets increased within a train (at OPT IDI) and increased with ITMS intensity (Fig. 3). The minimum ITMS intensity that resulted in an increase in MEP amplitude during a train of 4 doublets (delivered at OPT IDI) was on average  $111\% \pm 3\%$  RMT.

#### Baseline MEP Measurements

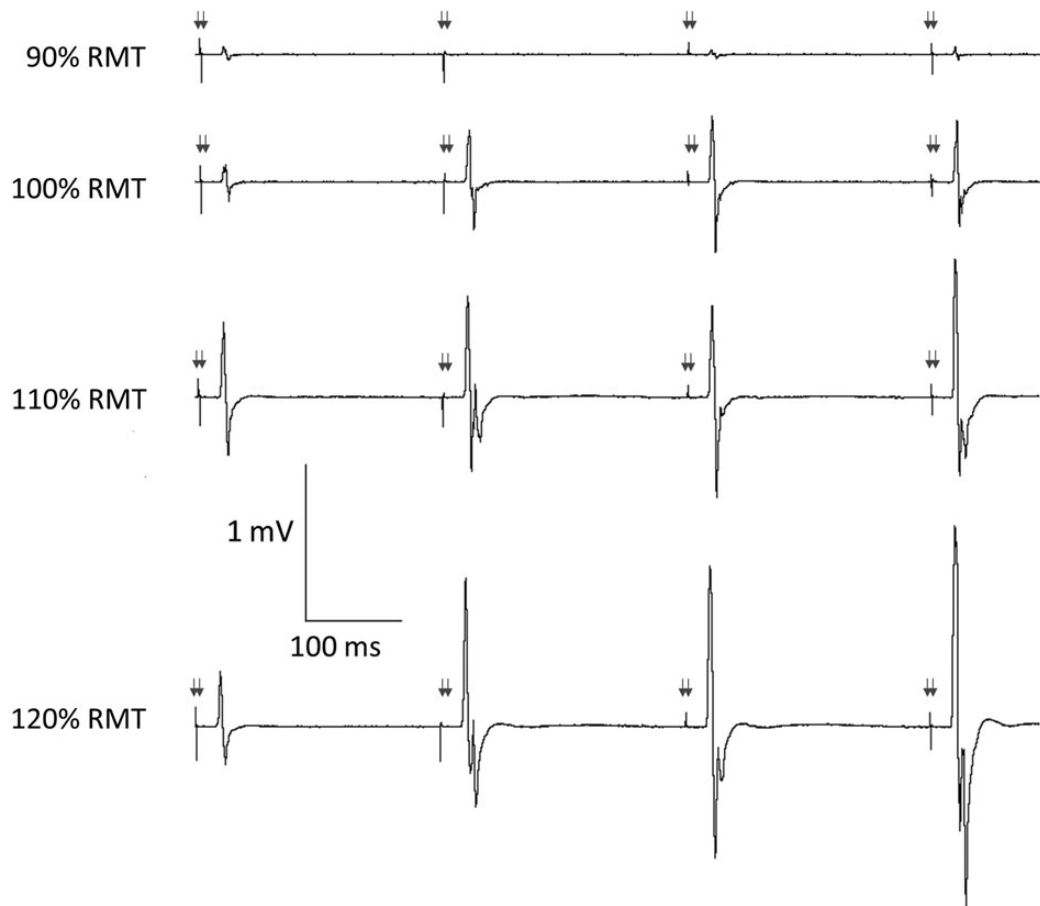
Baseline TS MEP amplitude (i.e.,  $SI_{1mV}$ ) did not differ for the 4 interventions ( $1.08 \pm 0.03$ ,  $0.97 \pm 0.07$ ,  $0.92 \pm 0.05$ , and  $0.97 \pm 0.1$  mV for OPT-SP, OPT-50, OPT, and OPT+50, respectively; LMM:  $F_{3,13.4} = 2.0$ ;  $P = 0.168$ ).

#### Changes in MEP Amplitude during Intervention

There was no significant difference within or across intervention in the MEP amplitude elicited by the first ITMS doublet of each of the 6 trains (LMM: effect of train:  $F_{5,37.2} = 0.427$ ,  $P = 0.83$ ; effect of intervention:  $F_{3,55.5} = 0.35$ ,  $P = 0.79$ ; interaction train  $\times$  intervention:  $F_{15,28.46} = 0.24$ ,  $P = 1.0$ ). SP MEP amplitude was successfully matched to MEP amplitude elicited by ITMS doublets ( $1.40 \pm 0.35$  mV vs.  $1.31 \pm 0.25$  mV, respectively). The ratio of MEP amplitude elicited by the fourth to the first ITMS doublet did not differ significantly for each train within any



**Figure 2.** (a) IPI curve for short-interval intracortical facilitation (SICF). The effect of IPI on SICF was significant (LMM:  $F_{6,14} = 3.8$ ;  $P < 0.05$ ). On average, SICF was maximal at an IPI of 1.3 ms, and SICF was significantly greater than SP baseline at IPIs of 1.1, 1.3, and 1.5 ms ( $256\% \pm 37\%$ ,  $339\% \pm 66\%$ , and  $276\% \pm 51\%$  of baseline, respectively;  $P < 0.05$ ). Individually, the maximum SICF occurred at an IPI of either 1.3 ms ( $n = 6$  participants) or 1.5 ms ( $n = 2$ ), and subsequently ITMS doublets were delivered at these individually optimal IPIs. (b) Interstimulus interval for maximum LICF. Triple-pulse TMS (see inset) was used to determine the temporal pattern of inhibition (equivalent to LICF) and LICF (it was previously shown that this MEP facilitation correlates with LCD) evoked by TMS doublets. Doublets were delivered at individual I-wave frequency of  $\sim 1.5$  ms (optimal interpulse interval, IPI-OPT) followed by a test stimulus (TS) at 150–300 ms. On average, the greatest reduction in TS MEP amplitude occurred 150 ms post-ITMS consistent with LICF ( $25\% \pm 16\%$  of baseline,  $P < 0.05$ ), and the greatest increase occurred at 220 ms ( $164\% \pm 30\%$  of baseline;  $P < 0.05$ ). The optimal IDI of LCD for each subject occurred at either 220 ms ( $n = 4$ ) or 250 ms ( $n = 4$ ) post-ITMS, and these values were used to set the IDI for each participant. In all cases, TS amplitude returned to baseline by 300 ms. (c) Effects of ITMS doublet intensity on LICF. At the individual optimal IDI (OPT, see inset), increasing the intensity of the pulses making up the ITMS doublet, from 90% to 120% RMT (in 10% steps), led to a progressive increase in TS MEP ( $90\% \pm 11\%$ ,  $122\% \pm 13\%$ ,  $175\% \pm 31\%$ , and  $263\% \pm 51\%$ , respectively, of TS baseline; LMM:  $F_{3,8.5} = 5.47$ ,  $P < 0.05$ ). Post hoc *t*-tests revealed that the minimum intensity for a significant increase in TS MEP amplitude (indicating LICF) was 110% RMT. \* $P < 0.05$ ; \*\* $P < 0.01$ ; results expressed as mean  $\pm$  SEM.



**Figure 3.** Influence of ITMS doublet intensity on MEP amplitude during train at OPT. MEPs during a 4-doublet train in 1 representative participant at the individual OPT (250 ms, arrows indicate approximate time of TMS doublets). When TMS doublets were delivered at 100% RMT, an intensity too low to generate LICF, there was no continuous increase in MEP amplitude throughout the train. However, MEP amplitude increased throughout the train at higher TMS doublet intensities of 110%–120% RMT, in line with the increase in LICF with TMS doublet intensity in Figure 2c.

intervention but did differ between interventions (LMM: effect of train:  $F_{5,33.9} = 0.4$ ,  $P = 0.85$ ; effect of intervention:  $F_{3,55.6} = 4.9$ ,  $P < 0.01$ ; interaction train  $\times$  intervention:  $F_{15,22.2} = 0.76$ ,  $P = 0.71$ ). Post hoc analyses indicated that this ratio was significantly greater for OPT ( $2.19 \pm 0.18$ ) compared with the other interventions ( $1.03 \pm 0.08$ ,  $1.34 \pm 0.05$ ,  $1.16 \pm 0.05$  for OPT – SP, OPT – 50, and OPT + 50, respectively;  $P < 0.01$  for each comparison with OPT, comparisons between control conditions not significant; Fig. 4).

#### Changes in MEP Amplitude Postintervention

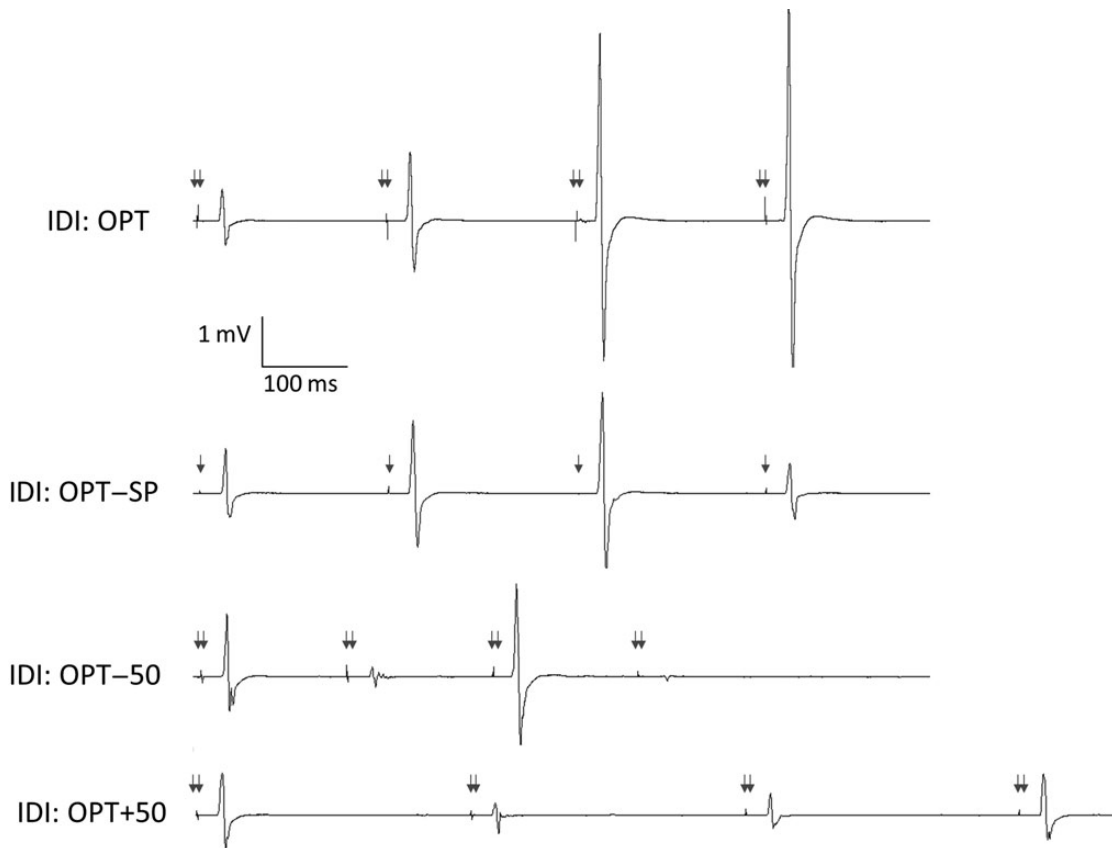
Figure 5 illustrates the time-course of MEP amplitude changes postintervention. There was a significant effect of time, intervention, and their interaction (LMM: effect of time:  $F_{10,19.4} = 14.1$ ,  $P < 0.001$ ; effect of intervention:  $F_{3,97.1} = 27.6$ ,  $P < 0.001$ ; interaction:  $F_{29,24.8} = 26.5$ ,  $P < 0.001$ ). Pairwise comparisons indicated that OPT differed significantly to the other interventional treatments (all comparisons  $P < 0.001$ ), whereas no differences between the other interventions were detected. Post hoc *t*-tests showed that MEP amplitude was increased at all time-points postintervention (1–31 min) for the OPT intervention, whereas no other intervention showed a significant MEP change (Fig. 5). The average MEP (derived from the group average) over 31 min postintervention relative to preintervention baseline was

$147\% \pm 4\%$  at OPT,  $105\% \pm 4\%$  at OPT – 50,  $108\% \pm 3\%$  at OPT + 50, and  $105\% \pm 5\%$  in the SP condition (inset, Fig. 5). There was no significant correlation between stimulus intensity and intervention efficacy at OPT ( $r = 0.48$ ,  $P = 0.23$ ). The interindividual variability of this MEP increase for the 4 interventional conditions is displayed in Figure 6, indicating consistent MEP increase across individuals at OPT and no change in the other conditions.

#### Experiment 2

##### SICF

The effects of IPI and muscle on SICF were significant (LMM: effect of IPI:  $F_{6,40.7} = 22.0$ ,  $P < 0.01$ ; effect of muscle:  $F_{3,113.2} = 32.9$ ,  $P < 0.01$ ). Peak SICF in the FDI target muscle was  $353\% \pm 52\%$  of TS alone, and in APB, ECR, and BB, it was  $600\% \pm 112\%$ ,  $316\% \pm 53\%$ , and  $153\% \pm 19\%$ , respectively, of TS alone (Fig. 7a). There was a trend for greater SICF in APB compared with FDI, but this difference was not significant. SICF was maximal at IPI 1.3 ms in all muscles (averaged across individuals). Mean test MEP amplitude (at 110% RMT) was  $0.60 \pm 0.12$  mV for FDI, and in APB, ECR, and BB, it was  $0.55 \pm 0.18$ ,  $0.25 \pm 0.06$  and  $0.1 \pm 0.02$  mV, respectively.



**Figure 4.** Influence of IDI on MEP amplitude during train. MEPs during a 4-doublet train in 1 individual. Doublets at the individualized OPT (220 ms) resulted in a continuous increase in MEP amplitude throughout the train that was not observed at other intervals (OPT – 50 ms, OPT + 50 ms) or when using matched amplitude single-pulse stimuli (OPT – SP). TMS intensity was 110% RMT; arrows indicate approximate time of TMS.

### LICF

There were significant effects of IDI and muscle on LICF (LMM: effect of IDI:  $F_{6,47.9} = 7.9$ ,  $P < 0.01$ ; effect of muscle:  $F_{3,39.3} = 9.4$ ,  $P < 0.01$ ). LICF at the OPT IDI was greatest for the FDI target muscle ( $196\% \pm 55\%$  of TS baseline,  $P < 0.05$ ) and decreased with increasing distance from the target muscle: APB:  $176\% \pm 36\%$  ( $P < 0.05$ ), ECR:  $114\% \pm 21\%$  (ns), and BB:  $101\% \pm 3\%$  (ns) of TS baseline (Fig. 7b). The MEP amplitude elicited by TS alone was  $1.15 \pm 0.15$  mV in FDI, and in APB, ECR, and BB, it was  $1.31 \pm 0.3$ ,  $0.41 \pm 0.01$ , and  $0.15 \pm 0.03$  mV, respectively. OPT IDI (maximum LICF) varied between 200 and 250 ms.

### Baseline MEP Measurements

Prior to intervention, baseline MEP amplitudes were  $0.81 \pm 0.08$  mV in FDI,  $0.78 \pm 0.24$  mV in APB,  $0.36 \pm 0.09$  mV in ECR, and  $0.14 \pm 0.04$  in BB.

### Changes in MEP Amplitude during Intervention

During intervention, the ratio of MEP amplitude elicited by the fourth to the first ITMS doublet differed between muscles (LMM: effect of muscle:  $F_{3,14.6} = 5.4$ ;  $P < 0.05$ ). Post hoc analyses indicated that the ratio was not significantly different between FDI ( $2.15 \pm 0.28$ ) and APB ( $1.93 \pm 0.32$ ) but was significantly greater in FDI (but not APB) compared with ECR ( $1.72 \pm 0.32$ ) and BB ( $1.15 \pm 0.12$ ), both comparisons  $P < 0.05$ . Average MEP amplitude of the first pulse in each train differed between muscles ( $P < 0.01$ ) but not across trains (LMM: effect of muscle:

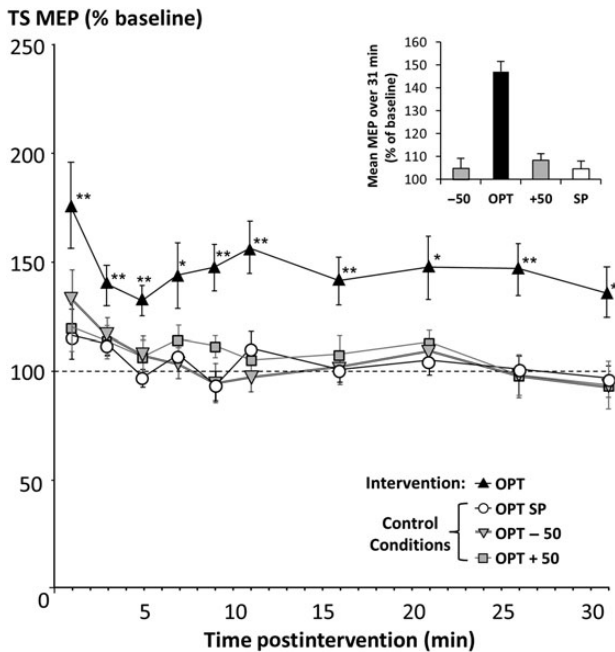
$F_{3,46.7} = 46.3$ ,  $P < 0.01$ ). This amplitude was  $1.83 \pm 0.10$  mV for FDI, and in APB, ECR, and BB, it was  $1.63 \pm 0.2$ ,  $0.61 \pm 0.01$ , and  $0.17 \pm 0.02$  mV, respectively.

### Changes in MEP Amplitude Postintervention

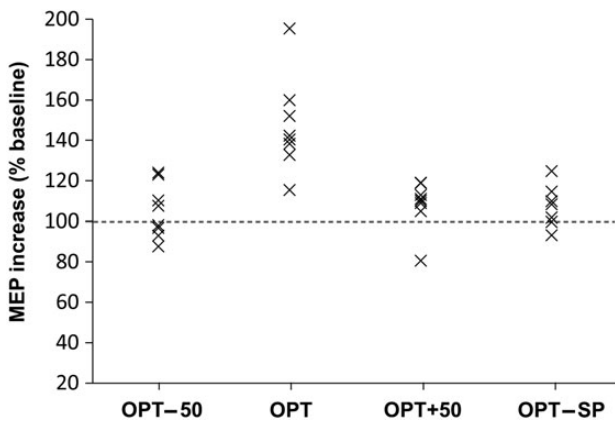
There was a significant effect of time, muscle, and their interaction (LMM: effect of time:  $F_{15,18.0} = 419.6$ ,  $P < 0.001$ ; effect of muscle:  $F_{3,76.3} = 24.4$ ,  $P < 0.001$ ; interaction:  $F_{43, 12.3} = 3.3$ ,  $P = 0.05$ ) (Fig. 8). Pairwise comparisons revealed that the response in both FDI and APB differed compared with the other muscles (all comparisons  $P < 0.05$ ) but did not differ significantly between FDI and APB. Post hoc *t*-tests revealed significant postinterventional increases in TS MEP amplitude compared with baseline for up to 60 min in FDI and APB (Fig. 8). The increase in MEP amplitude averaged over 120 min postintervention correlated with LICF across muscles ( $r = 0.88$ ,  $P < 0.05$ ; Fig. 9). Taken together, these results indicate topographic specificity of the intervention to the M1 hand representation stimulated by TMS.

### Discussion

When paired with LCD, just 48 TMS pulses delivered over less than a minute were sufficient to induce a long-lasting (up to 60 min) period of increased corticomotor excitability. There was no such effect at intervals not corresponding to LCD or when the ITMS doublets were replaced with SP TMS (adjusted in intensity to match MEP amplitude). The intervention was



**Figure 5.** Time-course of MEP amplitude changes postintervention. The primary TMS intervention at OPT resulted in a significant increase in MEP amplitude postintervention ( $P < 0.001$ ), whereas control experiments showed that IDIs shorter or longer than LCD did not result in LTP-like plasticity, indicating that LCD facilitates synaptic plasticity in human M1. The average MEP over 31 min postintervention relative to preintervention baseline was  $147\% \pm 4\%$  at OPT,  $105\% \pm 4\%$  at OPT - 50,  $108\% \pm 3\%$  at OPT + 50, and  $105\% \pm 5\%$  in the OPT - SP condition (see inset). \* $P < 0.05$ , \*\* $P < 0.01$  (post hoc  $t$ -tests, significant difference of TS MEP amplitude from baseline).



**Figure 6.** Interindividual variability. The average increase in excitability pre- to post-intervention (1–30 min, % baseline) is indicated for each individual and each interventional protocol. The increase in postintervention excitability was consistent across participants at OPT with little change at other intervals.

topographically specific, and its effectiveness was proportional to the level of LICF for each muscle. The results provide novel evidence that targeting disinhibition can be highly effective at inducing LTP-like effects in human motor cortex.

The period that corresponds to LCD is thought to arise from a disparity between the time-course of pre- and post-synaptic GABA<sub>B</sub>ergic inhibition (Otis et al. 1993; Deisz 1999). In the human, LCD can be identified in a modified LICI protocol in which the interstimulus interval is extended beyond the time-course of LICI. In this post-LICI period, the conditioned MEP amplitude is greater than the unconditioned MEP, resulting in

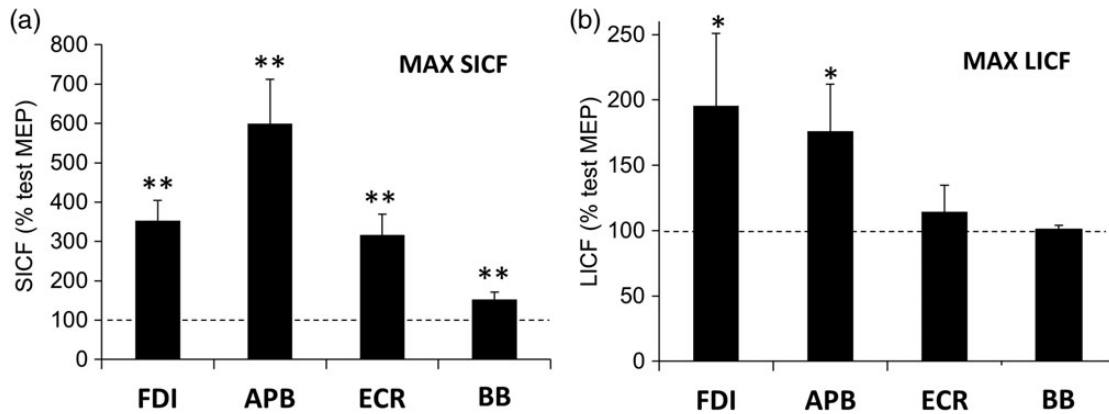
the phenomenon of LICF. This late-interval MEP facilitation is comparable with a postsynaptic depolarization that occurs during the late disinhibitory period in cellular studies (Larson and Lynch 1986; Pacelli et al. 1989). We have shown that during (but not after) LICF, inhibition (as determined by SICI) is decreased as would be expected from disinhibition (Cash et al. 2011). We therefore identified this period, which follows on from LICI and lasts ~50 ms, with LCD, and it is this period that we have targeted for the delivery of ITMS.

The intervention we chose to incorporate with LCD was repetitive ITMS. SICF circuitry underpins ITMS (Sewerin et al. 2011; Cash et al. 2013) and is enhanced during LCD (Cash et al. 2011), which provides a rationale for this combination. We also established here that SICF doublets evoke the same pattern of disinhibition (i.e., LICI followed by LICF, Fig. 2b) as was previously observed following a single stimulus (Cash et al. 2010, 2011). We decided to deliver the protocol at high frequency (i.e., trains of 4 doublets at individual LCD interval of 200–250 ms) to shorten the protocol duration to <1 min, and because previous protocols of 5 Hz paired-associative stimulation (Quartarone et al. 2006) and theta-burst stimulation, that is, burst stimulation at 5 Hz (Huang et al. 2005), demonstrated the feasibility to induce long-term changes in corticospinal excitability. We inserted an intertrain interval of 8 s for safety reasons as well as to avoid a potential buildup of inhibitory effects that can arise from continuous stimulation (Huang et al. 2005, 2011; Rothkegel et al. 2010).

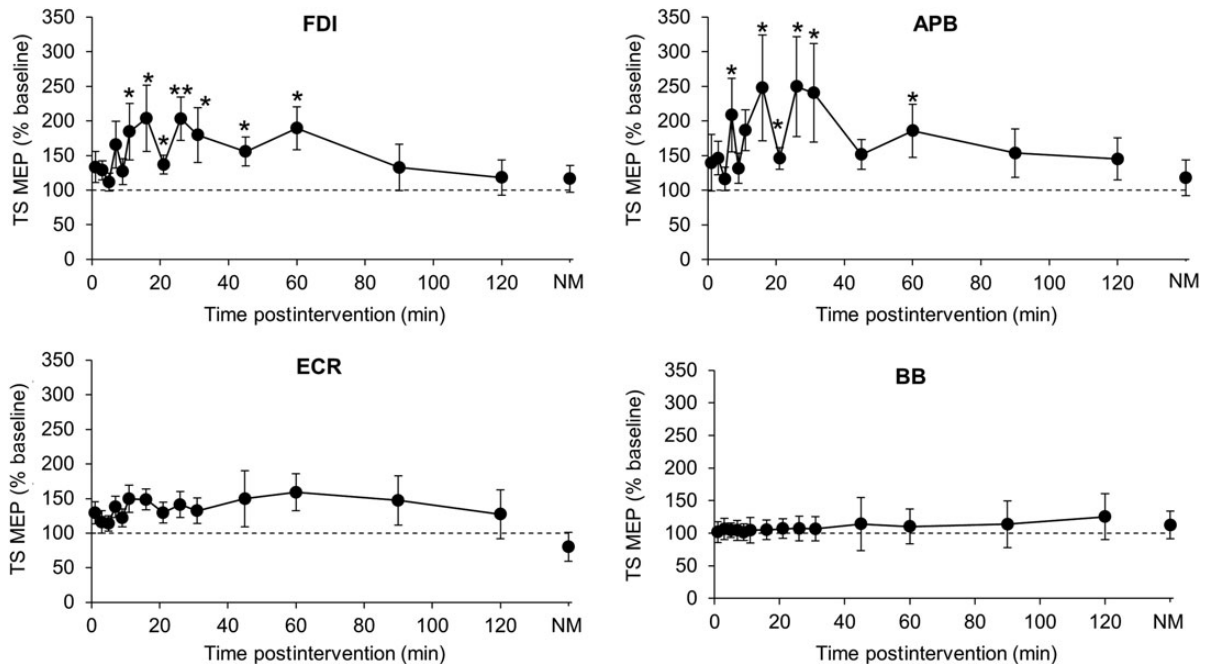
The DIS protocol contained a number of optimizations. While ITMS originally used a fixed IPI of 1.5 ms [corresponding to the first SICF peak (Thickbroom et al. 2006)], it has recently been shown that this interval is critical and that increasing the IPI to 2 ms (corresponding to the next SICF trough) results in LTD-like behavior (Cash et al. 2013). A better effect size has also been reported when the IPI is optimized for each individual according to peak SICF (Sewerin et al. 2011). It therefore appears that ITMS effects closely follow SICF phase dynamics (Cash et al. 2013). Given this sensitivity to IPI, we tailored IPI to each individual SICF peak. Likewise, as LCD covers a relatively short-time window that could be missed according to individual variation, we determined the optimal IDI according to the LCD peak in each individual. Intensity of stimulation was set based on a compromise between the intensity that gave higher LCD (i.e., higher intensity) and safety considerations. Finally, as the technique depended on both disinhibition and I-wave facilitation, the presence of LICF and SICF were inclusion criteria for the study, resulting in a small number of participants ( $5/22 = 22.7\%$ ) not proceeding past screening. For comparison, 15%–35% of healthy subjects do not show SICI or intracortical facilitation (Wassermann 2002; Orth et al. 2003). All of these considerations were included in the design to maximize the efficacy of the DIS protocol and minimize variability by accounting for individual physiological parameters.

Of note, SP TMS adjusted in intensity to match MEP amplitude elicited by TMS doublets did not result in LTP-like MEP increase when given at the optimal individual interstimulus interval to target LCD. This is an important control experiment that suggests that SICF, reflecting activation of glutamatergic excitatory cortical interneurons that are also responsible for the generation of I-waves (Ilic et al. 2002), and LCD, likely reflecting GABA<sub>B</sub>ergic disinhibition (Cash et al. 2011), cooperate for induction of LTP-like plasticity.





**Figure 7.** Topographic differences in SICF and LICF. (a) SICF and (b) LICF were calculated at the optimal IPI for FDI, APB, ECR, and BB muscles. Both measures were greatest in hand muscles. SICF was greatest in APB and FDI but was still significant in ECR and BB, whereas LICF was greatest in the FDI target muscle but not significant in ECR and BB. Results expressed as means  $\pm$  SEM. \* $P < 0.05$ ; \*\* $P < 0.01$  (post hoc  $t$ -tests, significant difference from test MEP amplitude).

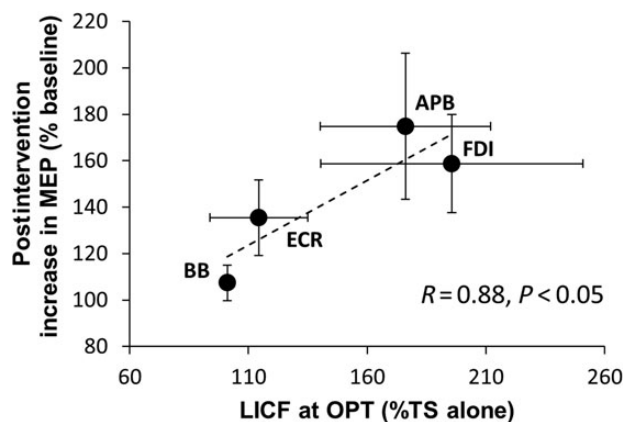


**Figure 8.** Topographic specificity of MEP amplitude changes postintervention. Comparison of the percentage change (compared with baseline) in single-pulse MEP amplitude 1–120 min and the next morning (NM) after intervention delivered at OPT to FDI hotspot, shown for FDI, APB, ECR, and BB muscles. LMM indicated a significant effect of time in FDI and APB, but not ECR or BB. Data are means  $\pm$  SEM. \* $P < 0.05$ ; \*\* $P < 0.01$  (post hoc  $t$ -tests, significant difference of TS MEP amplitude from baseline).

There is increasing interest in the basis for variation in the efficacy of most TMS interventions, and a number of factors are being considered including genetic polymorphisms (for review, [Ridding and Ziemann 2010](#)). Physiological parameters may be directly linked to genetic variation, for example, in the mouse, genetic deletion of the GABA transporter (GAT1), which is responsible for reuptake of GABA from the synaptic cleft, prolongs the decay kinetics of GABAergic transmission, decreasing theta oscillation frequency and modulating the frequency range in which theta-burst stimulation can induce LTP ([Gong et al. 2009](#)). Furthermore, genetic deletion of GABA<sub>B</sub> autoreceptors in *Gabbr1<sup>-/-</sup>* mice leads to a failure to exhibit LTP ([Vigot et al. 2006](#)). It is conceivable that interindividual

temporal variation in the latency or presence of LCD or variation in the efficacy of TMS plasticity protocols is linked to GAT activity or GABA receptor polymorphisms. Determining physiological variations between individuals and modifying individual parameters accordingly, where possible, or considering such factors in the choice of a suitable intervention for a given individual may enhance efficacy of plasticity induction. Other factors such as SICF IPI ([Sewerin et al. 2011](#); [Cash et al. 2013](#)) and stimulus intensity also may be important. We observed that stimulus intensity influenced the progressive increase in MEP amplitude during a train (Fig. 3), in line with intensity driven during- and post-train effects in animal studies ([Timofeev et al. 2002](#)). It has also been observed that reducing





**Figure 9.** Correlation between LICF and mean postintervention increase in MEP amplitude. Correlation of LICF (x-axis) and the mean increase in MEP amplitude 1–120 min postintervention over MEP amplitude at baseline (y-axis) for each muscle ( $r = 0.88$ ,  $P < 0.05$ ). Horizontal and vertical error bars indicate SEM for LICF and increase in MEP amplitude postintervention, respectively.

stimulus intensity can result in a switch from LTP- to LTD-like effects with human regular rTMS and theta-burst stimulation protocols (Berger et al. 2011; Doeltgen and Ridding 2011). It remains to be seen whether stimulation during LCD can enhance LTD-like plasticity; cellular studies have demonstrated that pharmacological GABAergic disinhibition resulted in abolition of LTD (Watanabe et al. 2007) or even a switch toward induction of LTP (Caballero et al. 2014). The protocol could be modified in a number of ways to induce LTD-like decreases in excitability such as by stimulation during the LICF phase, continuous stimulation akin to continuous theta-burst stimulation, lower intensity, or targeting the SICF trough of 2 ms. Examining this broad range of possibilities will be of interest in future studies.

The extent to which the approach might generalize to other excitatory TMS interventions would also be of interest. In principle, theta-burst stimulation (Huang et al. 2005), paired-associative stimulation (Stefan et al. 2000), and quadropulse stimulation (Hamada et al. 2007) could be adapted to conform to LCD dynamics. Indeed, it has been previously suggested that the effectiveness of intermittent theta-burst stimulation may be due to a serendipitous similarity between theta rhythm periodicity (200 ms) and LCD (Thickbroom 2007), and such a connection has been proposed in cellular studies (Larson et al. 1986; Davies et al. 1991; Mott and Lewis 1991). In the present study, a  $\pm 50$ -ms adjustment in periodicity resulted in a significant loss of effect size, suggesting that tuning theta-burst stimulation frequency to match LCD periodicity may be an option and could potentially help to reduce variability between individuals, which has recently become a topic of considerable interest (Hamada et al. 2013). We also observed a relatively low level of variability in plasticity response (Fig. 6), which may be attributable to adjusting for individual variation in physiological parameters, controlling for factors such as time of day, which can influence cortisol levels (Sale et al. 2007, 2008) and GABAergic inhibition in motor cortex (Lang et al. 2011), avoiding testing at certain timing during the menstrual cycle (Inghilleri et al. 2004), which can affect plasticity, and asking participants to avoid contraction during- and post-intervention, which has been associated with depotentiation (Gentner et al. 2008; Huang et al. 2008).

Increases in excitability postintervention were specific to the M1 hand representation stimulated by TMS. The postintervention MEP amplitude increase in each muscle was proportional to the degree of LICF (Fig. 9). Similar topographic specificity has been shown in other studies using noninvasive brain stimulation for LTP-like plasticity induction (Stefan et al. 2000; Ridding and Taylor 2001; Rosenkranz and Rothwell 2006; Cheeran et al. 2008; Pötter-Nerger et al. 2009; Dileone et al. 2010; Ni et al. 2014), and for interneuronal circuitry (Shimizu et al. 1999; Chen and Garg 2000; Saisanen et al. 2011).

The aftereffect we observed by targeting disinhibition was long-lasting with respect to the duration of the intervention. It is known that disinhibition can have a powerful effect on plasticity induction, even to the extent of being permissive (Davies et al. 1991; Mott and Lewis 1991; Hess and Donoghue 1994), or evoking LTP ( $\sim 30$  min) from a single primed burst (Diamond et al. 1988) or single non-primed burst in the presence of a GABA<sub>A</sub> receptor antagonist (Wigstrom and Gustafsson 1983a, 1985). Despite this strong aftereffect at OPT, during the intervention, there was no statistical increase in MEP amplitude for successive trains, although MEP amplitude within each train increased. It is possible that LTP-like effects take some time to manifest and that they may not become apparent until after the intervention, given that the duration of the intervention was  $< 1$  min. One advantage to the absence of a within-intervention effect is that it may reduce the likelihood of a spreading increase in corticomotor excitability that may develop rapidly during high-frequency regular rTMS and would constitute a safety concern (Pascual-Leone et al. 1993). Disinhibition at the cellular level has been shown to permit the depolarization necessary to adequately unmask the NMDAR ionophore from Mg<sup>2+</sup> blockade, thus facilitating NMDAR-mediated Ca<sup>2+</sup> current during subsequent stimulation and resulting in greater plasticity induction (Larson and Lynch 1986; Mott and Lewis 1992; Davies and Collingridge 1996), with NMDAR currents accounting for  $\sim 35\%$  of the increase in response amplitude during a disinhibited burst train (Larson and Lynch 1988). Indeed, one of the proposed physiological effects for presynaptic disinhibition is to facilitate the synaptic activation of NMDA receptors during trains (Collingridge et al. 1988; Mangan and Lothman 1996). Similar mechanisms may contribute to the increase in MEP amplitude during the trains and induction of LTP-like plasticity in the present OPT protocol. As local Ca<sup>2+</sup> transients decay over several hundred milliseconds, NMDAR activity may be progressively more easily evoked during a disinhibited train but may decline between trains if the intertrain interval is several seconds long (Maeda et al. 1999; Koester and Sakmann 2000; Wittenberg and Wang 2006). Such properties of Ca<sup>2+</sup> transients could potentially account for the progressive increase in MEP amplitude during the train but not from one train to the next. The steady increase in MEP amplitude within a train could also suggest that successive ITMS pairs continue to evoke and progressively build up LCD. We have previously argued that disinhibition may have a role in entraining cortical rhythms (Cash et al. 2010, 2011), which could occur if successive phases of disinhibition were sustained within a target network, or if after-hyperpolarization rebound currents interact with disinhibition.

Pharmacological disinhibition is not available for use in humans because GABA receptor antagonists have proconvulsant properties. Motor cortical disinhibition evoked by transient deafferentation using limb ischemic nerve block (Brasil-Neto

et al. 1993; Levy et al. 2002) or selective upper brachial plexus anesthesia enhances LTP-like plasticity and motor learning in healthy subjects (Ziemann, Corwell et al. 1998, 2001) and in chronic stroke patients (Muellbacher et al. 2002), but these techniques are painful and too complex so that they have not gained wide application. The present method of electrophysiologically evoking disinhibition in M1 offers an elegant alternative requiring only 1 IPI curve to identify LCD and could be considered for incorporation into other plasticity protocols.

Finally, as a limitation of the current study, we have not proven the cortical origin of the LTP-like MEP increase. However, we have shown previously that ITMS results in an increase in SICF (Cash et al. 2009), which reflects excitability of neural elements in motor cortex responsible for I-wave generation (Ilic et al. 2002). Plasticity induction was also dependent on delivery during a time window (LCD) that we have previously shown involves cortical interneuronal SICI and SICF circuitry. The facilitation of SICF during LCD also suggests that the cortical circuitry targeted by ITMS is facilitated during this period. Therefore, we consider it very likely that the LTP-like MEP increase in the present modified ITMS experiment also originated in motor cortical I-wave circuitry. Another limitation is that we have not proven that a doublet PS is necessary for induction of LTP-like plasticity. This would require substitution of the doublet PS by an SP PS paired with a doublet TS at the OPT interval. However, testing this in the high-frequency trains of the present experiment is not feasible because subsequent alternating SP PS and doublet TS in the train interact with each other. Therefore, repeated application of this SP PS + doublet TS pair at low frequency (e.g., 0.2 Hz for 15 min, as in standard ITMS protocols) would be mandatory. As we aimed here at creating a brief protocol (<1 min) for highly effective LTP-like plasticity induction, such an additional experiment would be beyond the intent and scope of the present study. Finally, the OPT – SP condition was matched to the OPT condition with respect to initial MEP amplitude in the trains. While it could be argued that a perfect match would be one in which the OPT – SP condition also resulted in a within-train increase in MEP amplitude, this would have been impractical to implement given the variability between trains and individuals. The current experiments suggest that a within-train MEP increase during intervention may be important for induction of LTP-like plasticity. Lastly, although we refer to the changes as “LTP-like” due to several shared cardinal characteristics including duration of  $\geq 30$  min, topographic specificity, and associativity, it is not possible to confirm that LTP has occurred using noninvasive methods at the systems level in human subjects.

In conclusion, the results indicate that by applying patterned stimulation that evokes both disinhibition and synaptic plasticity (DIS), a long-lasting aftereffect can be induced by a remarkably short period of stimulation. The ability to evoke disinhibition in human motor cortex offers a powerful new approach for modulating corticomotor excitability and plasticity induction with noninvasive brain stimulation. By harnessing this physiological effect, it may be possible to further improve the delivery and efficacy of neuromodulatory protocols in clinical applications.

### Supplementary Material

Supplementary material can be found at: <http://www.cercor.oxfordjournals.org/>.

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### Notes

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