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Combined endogenous and exogenous disinhibition of intracortical circuits augments plasticity induction in the human motor cortex

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ABSTRACT

Background: Motor imagery (MI) engages cortical areas in the human brain similar to motor practice. Corticospinal excitability (CSE) is facilitated during but not after MI practice. We hypothesized that lasting CSE changes could be achieved by associatively pairing this endogenous modulation with exogenous stimulation of the same intracortical circuits.

Methods: We combined MI with a disinhibition protocol (DIS) targeting intracortical circuits by paired-pulse repetitive transcranial magnetic stimulation in one main and three subsequent experiments. The follow-up experiments were applied to increase effects, e.g., by individualizing inter-stimulus intervals, adding neuromuscular stimulation and expanding the intervention period. CSE was captured during (online) and after (offline) the interventions via input-output changes and cortical maps of motor evoked potentials. A total of 35 healthy subjects (mean age 26.1 ± 2.6 years, 20 females) participated in this study.

Results: A short intervention (48 stimuli within ~90s) increased CSE. This plasticity developed rapidly, was associative (with MI_{on}, but not MI_{off} or REST) and persisted beyond the intervention period. Follow-up experiments revealed the relevance of individualizing inter-stimulus intervals and of consistent inter-burst periods for online and offline effects, respectively. Expanding this combined MI/DIS intervention to 480 stimuli amplified the sustainability of CSE changes. When concurrent neuromuscular electrical stimulation was applied, the plasticity induction was cancelled.

Conclusions: This novel associative stimulation protocol augmented plasticity induction in the human motor cortex within a remarkably short period of time and in the absence of active movements. The combination of endogenous and exogenous disinhibition of intracortical circuits may provide a therapeutic backdoor when active movements are no longer possible, e.g., for hand paralysis after stroke.

Introduction

Reorganization and repair of the lesioned brain are determined by experience-dependent plasticity [1]. In the motor system, plastic reorganization is predominantly driven by physical practice. Active movements might, however, no longer be possible following, for example, a severe stroke. Such conditions therefore necessitate therapeutic interventions which facilitate plasticity in a context that resembles physical practice. Current protocols for plasticity induction are, however, usually applied in a state of rest.

From a neurophysiological perspective, neuroplasticity is a largely stimulus-dependent synaptic phenomenon [2,3]. The capacity for plasticity is relevantly determined by the balance between gamma-Aminobutyric acid (GABA)-ergic inhibition and glutamatergic excitation within intracortical circuits [4]. Shifting this balance away from inhibition, i.e., disinhibition [5], facilitates neuroplasticity which can be captured in the motor system as long-term potentiation (LTP) of corticospinal excitability (CSE); modified CSE may be non-invasively indexed by transcranial magnetic stimulation (TMS)-induced changes of motor evoked potential (MEP) amplitudes [6].

Different TMS protocols may also be applied for the induction of plasticity, e.g., repetitive TMS pulses with a fixed frequency (rTMS) [7], patterned theta burst stimulation (TBS) [8], and associative pairing of cortical and peripheral stimuli (PAS) [9], to name a few (for an overview, see Ref. [10]). A novel paired-pulse TMS protocol,

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referred to as disinhibition stimulation (DIS), was recently reported to be highly effective in inducing LTP-like plasticity of CSE [11]. The effects were achieved by applying a remarkably short period of stimulation (i.e., a total of 48 stimuli within ~1 min), rendering this intervention particularly suitable for clinical application. DIS evoked both synaptic plasticity and disinhibition by specifically timing the interpulse interval (IPI) of a pair of stimuli (1.3–1.5 ms) as well as the interdoublet interval of two paired pulses (IDI; 200–250 ms), which probably reflect a cooperative effect of glutaminergic short-interval intracortical facilitation (SICF) [12–14] and $GABA_B$ergic late cortical disinhibition (LCD) [15,16] of I-wave generating neurons [15,17,18], respectively. During the exogenous disinhibition, a reduction in GABAergic short-interval intracortical inhibition (SICI) and an increase in SICF could be demonstrated [12,13,15]. Furthermore, DIS has been associated with an increased CSE, as reflected by increased MEP [11]. However, this exogenous disinhibition protocol has been applied at rest but not during tasks resembling physical practice.

Like actual motor practice, motor imagery (MI) engages motor cortical areas via, for example, sensorimotor event-related desynchronization (ERD) [19–24]. Specifically, kinesthetic MI, which involves imagining the feeling produced by actual task performance [25], induces a spatial and temporal modulation of motor cortical function that mirrors the modulation observed during actual motor practice [26]: Facilitation of CSE occurs at the time of imagined movements (ON phase), not between them (OFF phase). When combined with neuromuscular electrical stimulation (ES), MI enhanced ERD [27] and increased CSE to a larger extent than MI itself, thus reaching levels similar to those occurring during voluntary muscular contraction [28]. The MI-induced increases of CSE were related to intracortical processes mediated via both $GABA_A$ergic [29–31] and $GABA_B$ergic disinhibition [32], indicated by changes in SICI and LCD. However, the effects of such endogenous disinhibition of the I-wave generating neurons usually do not last longer than the intervention itself [33–35].

In this study with healthy subjects, we investigated a novel associative stimulation protocol by targeting intracortical circuits in a context that resembles physical practice, i.e., during kinesthetic MI. Due to their modulation of the same intracortical circuitries, we hypothesized that the combination of endogenous disinhibition by MI of finger extension and exogenous disinhibition (DIS) by paired-pulse rTMS of the respective cortical motor representation induces associative plasticity that lasts beyond the intervention. In this main experiment (Experiment 1), associativity was investigated with concurrent (MI_{ON}), delayed (MI_{OFF}) and independent (REST) DIS relative to the MI task. In the course of follow-up experiments (Experiments 2–4) that were applied to increase effects we also investigated the modulatory influence of individually adjusted IPI and IDI intervals, of concurrent neuromuscular electrical stimulation (ES) to the targeted muscle (Experiment 2), of an expanded intervention period (480 instead of 48 stimuli, Experiment 3) and of brain state-dependent stimulation (Experiment 4).

Materials and methods

Study design

A total of 35 healthy subjects (mean age 26.1 ± 2.6 years, range 20–35 years, 20 females) participated in the study, which consisted of a combined MI/DIS experiment and three follow-up experiments (see below). All subjects gave their written informed consent prior to participation in the study, which had been approved by the local ethics committee. The study was carried out in accordance with the latest version of the Declaration of Helsinki. The follow-up experiments were separated by at least four weeks and designed on the basis of the findings in the previous experiments to explore modulating factors and maximize CSE increases. In each experiment, four to five conditions were investigated in randomized order and separated by at least two days to prevent carry-over effects. Subjects were not informed as to the purpose and hypothesis of each experiment. All sessions were conducted at a similar time of day to minimize the effect of circadian fluctuations due to cortisol on CSE [36]. The subjects had no contraindications to TMS [37] nor had they any history of psychiatric or neurological disease. Right-handedness was confirmed by the Edinburgh handedness inventory [38].

A general overview of the experimental designs is provided in Fig. 1.

Experiment 1: associative combination of MI and DIS

For the main Experiment 1, a DIS protocol [11], specified in the following paragraphs, was paired with MI of finger and wrist extension [39] (for details see below). Four stimulation doublets (i.e., eight stimuli) were applied at the time of imagined movement (during the MI_{ON} phase). In all, 48 stimuli were applied during six MI trials (MI/DIS condition). To determine the associativity of the intervention, the same DIS protocol was applied during the MI_{OFF} phase, i.e., *after MI* (MI_{OFF}/DIS condition), or during REST, i.e., *without* MI (DIS condition). Moreover, the six MI trials were also performed *without* any DIS (MI condition). In summary, Experiment 1 consisted of four conditions (i.e., MI/DIS, MI_{OFF}/DIS, DIS, and MI) which were investigated on different days in randomized order.

Experiment 2: combination of MI/DIS with neuromuscular ES

The conditions of the follow-up Experiment 2 were similar to those in Experiment 1, but with simultaneous ES application (for details see below). ES was included in the experimental design since it was shown to increase the effects of single TMS pulses on CSE [40,41]. It was also shown to enhance MI-related ERD [27] and increase CSE to a greater extent than MI itself [28]. In summary, Experiment 2 consisted of four conditions (i.e., MI/DIS/ES, MI_{OFF}/DIS/ES, DIS/ES, and MI/ES) which were investigated on different days in randomized order.

Experiment 3: expanded intervention period

The conditions of the follow-up Experiment 3 were similar to the conditions in the main Experiment 1 (MI/DIS condition), but with a 10x expanded intervention period of 60 (instead of 6) trials and 480 (instead of 48) stimuli. We included this adjustment in the experimental design, since the intervention duration of paired-pulse rTMS is known to influence CSE [42]. The MI_{OFF}/DIS condition was not further considered in Experiment 3 due to the negative findings in Experiments 1 and 2. In summary, Experiment 3 consisted of four conditions (i.e., MI/DIS, MI/DIS_{10}, DIS_{10}, and MI_{10}) which were investigated on different days in randomized order.

Experiment 4: state-dependent stimulation

The conditions of the follow-up Experiment 4 were as in Experiment 2 (MI/DIS/ES condition), but with the 10x expanded number of cortical stimuli (480 pulses), thus matching the number of stimuli in Experiment 3. In addition, DIS and ES were applied subsequently instead of simultaneously, i.e., DIS + ES or ES + DIS. This modification of the study design was carried out on account of the negative findings of the simultaneous DIS/ES application in Experiment 2 and previous research on subsequent PAS [41,43]. In one further condition (MI/DIS_{D10}), the timing of DIS was *delayed* to

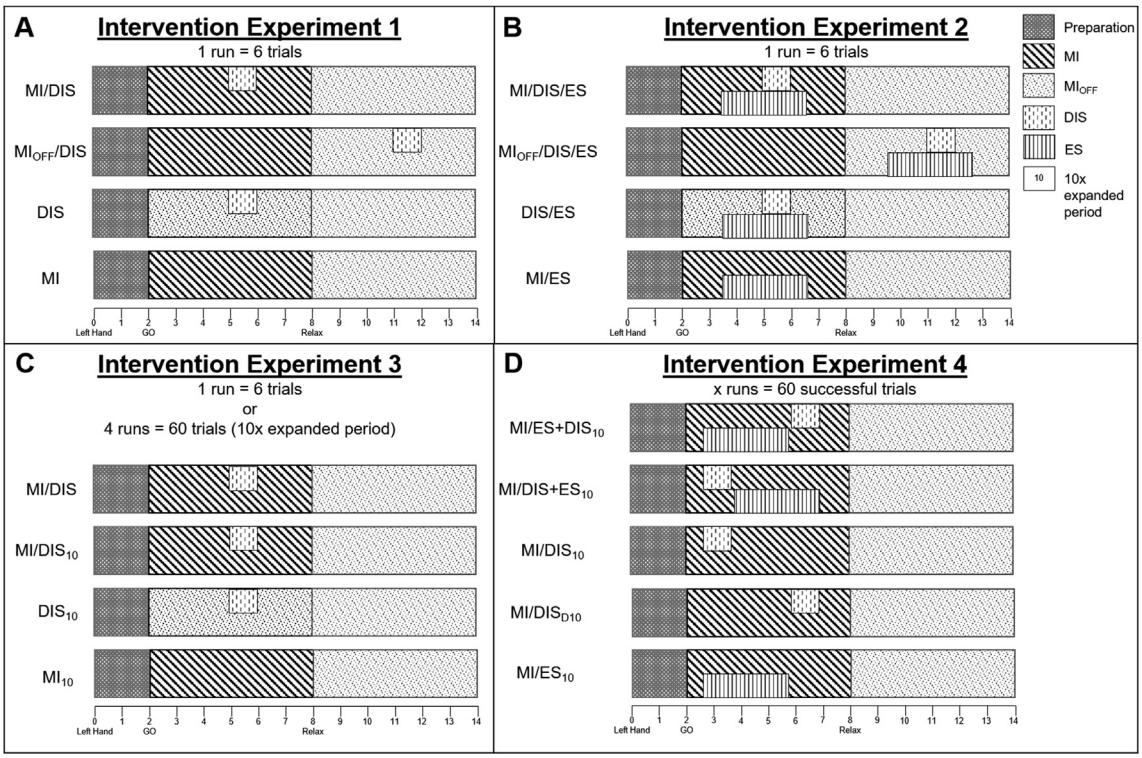

Fig. 1. Study design showing the building blocks of each experiment. A: Experiment 1 consisted of four conditions with one run containing six trials. DIS was paired with MI of finger/wrist extension (MI/DIS). As controls, DIS was applied during the MIOFF phase (MIOFF/DIS) or during REST (DIS). Furthermore, the six MI trials were performed without any DIS (MI). B: The follow-up Experiment 2 consisted of four conditions: MI/DIS was combined with ES (MI/DIS/ES). As controls, MIOFF/DIS, DIS, or MI were combined with ES (MI/DIS/ES, MIOFF/DIS/ES, DIS/ES, or MI/ES, respectively). Each condition had one run containing six trials. ES lasted 3 s and the DIS was applied halfway through the trial. C: The follow-up Experiment 3 consisted of four conditions that investigated the MI/DIS condition of Experiment 1 with a 10x expanded intervention period: MI/DIS, MI/DIS10, DIS10, and MI10. MI/DIS had one run containing six trials. The other conditions consisted of four runs with altogether60 trials, i.e., 15 trials per run. D: The follow-up Experiment 4 consisted of five previously studied conditions which were now triggered by event-related desynchronization: MI/ES + DIS10, MI/DIS + ES10, MI/DIS10, MI/DISD10 and MI/ES10. Stimulation bursts were applied only when a predefined ERD threshold was achieved until 60 successful trials had been obtained. In one additional condition (MI/DISD10), the timing of DIS was delayed to match the timing of DIS during the ES + DIS condition.

match the timing of DIS during the ES + DIS condition and to avoid a timing-dependent bias. Furthermore, on the basis of recent findings of state-dependent interventions [39,44], stimulation bursts were applied only when a predefined ERD threshold (for details see below) was achieved during MI so as to improve the associativity with MI-related ERD. In summary, Experiment 4 consisted of five conditions (i.e., MI/DIS$_{10}$, MI/DIS$_{D10}$, MI/ES$_{10}$, MI/ES + DIS$_{10}$, MI/DIS + ES$_{10}$) which were investigated on different days in randomized order.

Data acquisition

The methods applied in this study were identical to those of previous studies [39,45] and have been cited accordingly:

Electromyography (EMG) and/or electroencephalography (EEG) data were recorded (BrainAmp Amplifier) at a sampling rate of 5 kHz using an antialiasing band-pass filter with cutoff frequencies at 0.16 Hz and 1 kHz [39]. Impedances at all electrodes were kept below 10 kΩ. In a next step, data were transferred for immediate analysis to MATLAB (R2017a, The MathWorks, Inc., United States), where they were stored for more detailed analysis [39,46].

Ag/AgCI AmbuNeuroline 720 wet gel surface electrodes (Ambu GmbH, Germany) were used to record electromyography (EMG) activity from the left Extensor Digitorum Communis (EDC) muscle. Two electrodes were placed on the muscle belly 2 cm apart from each other.

In Experiment 4, Ag/AgCl electrodes (BrainCap for TMS, Brainproducts GmbH, Germany) were used to record EEG in a 64 channel setup that complied with the international 10–20 system (with FCz as reference, and AFz as ground) to allow for brain state-dependent stimulation (see below).

TMS protocol

Subjects were seated comfortably in an armchair with their elbow semi-flexed; the forearm was pronated, fully relaxed, and supported by the arm of the chair. The representation of the left EDC in the right M1 was determined for each subject prior to the onset of the experimental session [48,49]. TMS was delivered using a biphasic pulse (MagPro-R30 + MagOption, MagVentureGmbH, Germany) through a figure-of-eight coil (MCF-B70) at an orientation of 45° to the sagittal plane; the induced current was directed posterior to anterior in the first phase, and anterior to posterior in the second phase of the stimulus. Frameless stereotactic neuronavigation (TMS Navigator, Localite GmbH, Germany) utilizing a template MRI (MNI ICBM152 non-linear symmetric T1 average brain) was applied to support the localization of the TMS target position [47]. We used 40% of maximum stimulator output as the initial intensity applied to the 'hand knob' of M1 as approximated by the ICBM152 template. Whenever the initial stimulator output did not suffice to elicit MEPs, we increased the output in steps of 5%. We defined the coil site that consistently elicited the largest MEPs as our stimulation site. Having identified this 'hotspot', we then determined the resting motor threshold (RMT) by the relative frequency method, i.e., by detecting the minimum stimulus intensity that resulted in MEPs >50 μV in the peak-to-peak amplitude in at

least 5 out of 10 consecutive trials [50]. For Experiments 2–4, we investigated the added benefit of individualizing the DIS intervals, i.e., by determining the most effective IPIs and IDIs for each subject individually to maximize MEP amplitudes. In order to define the optimal interval for SICF and LCD, IPIs of 1.1, 1.2, 1.3, 1.4, and 1.5 ms, IDIs of 150, 200, 220, 230, and 250 ms, and the conditioning stimulus alone were each investigated ten times at the beginning of every experiment [11]. These eleven blocks were applied in a pseudo-randomized order.

General study design of the interventions

In Experiments 1 and 2, each condition consisted of one run with six trials. In Experiment 3, the expanded conditions consisted of four runs, each of which contained fifteen trials, resulting in 60 trials (i.e., 10x the trial number of Experiments 1 or 2). In Experiment 4, at least four runs, each consisting of fifteen trials, were performed until 60 trials (as in Experiment 3) with sufficient ERD and triggered stimulation had been obtained (Fig. 1D). In summary, both the Experiments 1 and 2 (48 stimuli), and the Experiments 2 and 3 (480 stimuli) had the same number of cortical stimuli.

In all experiments, each trial began with a 2 s preparation period, followed by a 6 s period of MI of finger/wrist extension of the left hand, and a 6 s MI_{OFF} period. The onset of the preparation, MI and MI_{OFF} periods were indicated by the auditory cue 'left hand', 'go' and 'relax', respectively. Subjects were instructed to perform kinesthetic MI during the MI period, i.e., to imagine a finger/wrist extension as accurately as possible by focusing on the sensory information, and to relax during the other periods [41,51–54]. For the conditions that combined MI and ES, participants were instructed to continue with MI when ES commenced, since previous work indicated that ERD increased when MI and ES occurred simultaneously, but not when the latter was triggered by the former [27].

Disinhibition stimulation (DIS)

Cortical stimulation was performed on the basis of a paired-pulse rTMS protocol, i.e., DIS [11]. DIS consisted of a train of four biphasic TMS doublets (eight pulses) at 110% RMT. In Experiment 1, the applied interpulse interval (IPI; 1.3 ms) of a doublet as well as the interdoublet interval (IDI; 220 ms) were predefined for all subjects on the basis of previous findings [11]. In the follow-up Experiments 2–4, the IPIs and IDIs to induce SICF and LCD, respectively were individually adjusted for each experimental session to enhance the impact of DIS on CSE [13]. In Experiments 1–3, DIS was initiated 3 s after the 'go' cue during the MI period, since the strongest ERD was detected at this point in time in our previous work [41,52–57]. For those conditions consisting of stimulation during the MI_{OFF} period, DIS was initiated 3 s after the 'relax' cue during the MI_{OFF} period. This control condition is characterized by low ERD or even event-related synchronization (ERS) [39]. For conditions consisting of stimulation during REST, DIS was initiated at the same point in time as during the MI condition, but the subjects did not perform MI. This ensured that the same number and pattern of cortical stimuli were applied in all conditions. In Experiment 4, the stimulation was triggered only if an ERD was observed (see following paragraphs).

Neuromuscular electrical stimulation (ES)

In Experiments 2 and 4, a 3 s ES train was applied to the left EDC muscle with a 1 ms pulse width at a frequency of 100 Hz (RehaStim 2 + stimulator, HasomedGmbH, Germany). Maximum ES intensity was individually adjusted to achieve complete finger and wrist extension, resulting in a mean of 7.7 ± 3.5 mA. Each ES train was 3 s long and included a 0.5 s ramp on/off phase. In Experiment 2, ES was initiated 1.5 s after the 'go' cue during the MI period. In Experiment 4, ES was triggered only if an ERD was observed (see next paragraph).

ERD detection

In Experiment 4, the stimulation (DIS/ES; ES/DIS; DIS) was triggered only if an ERD was observed in the β-band (16–22 Hz) during the MI phase [58]. We used a linear classifier of nine features consisting of three 2-Hz frequency bins (16–22 Hz) and three channels (FC4, C4, and CP4 over the right sensorimotor area [59]) to detect decreases in sensorimotor rhythm power in the β-band. This frequency band was selected on the basis of previous work in our group on beta-band oscillatory circuits in the extended motor network [45,60,61]. An autoregressive model, with a model order of 32 and based on the Burg Algorithm, was used to estimate frequency power [62]. Five consecutive 40 ms epochs (i.e., 200 ms) had to be classified as ERD-positive before stimulation could be initiated. This ensured that stimulation occurred during prolonged sessions of ERD only [39]. Prior to the experiment, a desynchronization task, consisting of three motor imagery training runs without stimulation, was performed for calibration to account for each subject's ability for desynchronization. Following this calibration session, an individual desynchronization threshold, described in detail elsewhere [63,64], was implemented for the intervention. This threshold balanced challenge and motivation of the participant and preserved the specificity of the feedback, i.e., stimulation was not provided until subjects attained consistent ERD. Stimulation did not occur in cases where the threshold had not been met due to ERS or when the ERD was not consistent, i.e., not long and/or not strong enough [39,55,58,60,65–68]. The ERD threshold ensured that each subject received the same task-related demand and that this remained constant throughout the intervention.

MEP amplitudes during the intervention (online)

MEP amplitudes elicited by doublet stimulation were measured to assess changes in CSE during the intervention induced by the condition. Due to the stimulation artifact during ES, DIS conditions containing simultaneous ES could not be analyzed with regard to MEP amplitudes during the intervention.

MEP amplitudes after the intervention (offline)

To study CSE before and after the interventions, TMS pulses were triggered every 5 s (±1.25 s predefined jitter).

For Experiments 1–4, we tested the MEP stimulus-response curve using a range between 90 and 150% RMT in 10% steps to determine CSE at baseline (prior to intervention) and after the intervention. This resulted in seven blocks of ten stimuli applied with seven different intensities; these blocks were applied in a pseudo-randomized order. In detail, for Experiments 1 and 2, post intervention measures were performed 15 and 60 min after the intervention. For Experiments 3 and 4, the post-measurement of the MEP stimulus-response curve was performed 15 min after the intervention only (but followed by CSE evaluation at 110% RMT and at shorter time intervals). More, specifically, on the basis of the results from Experiments 1 and 2 with regard to the stimulus-response curve and the cortical map (which was acquired at 110% RMT), lasting changes of CSE in Experiments 3 and 4 were tested at 110% RMT and at shorter time intervals to investigate the time-dependent changes of plasticity in more detail. In Experiment 3 and 4, this 110% RMT measurement was performed at 15, 30, 45, and 60 min and at 15, 30, 40, 50, and 60 min post-intervention, respectively.

Furthermore, we acquired a cortical map representation at 110% RMT for a virtual grid prior to and 30 min after the intervention. In Experiment 1, a 7-by-7 grid (5×5 mm per cell) was predefined in the navigation software. Three stimuli were applied at each grid cell (12 stimuli per cm^2) and the cortical map was extended in a circular manner by each grid point until all points were stimulated. Since we could still induce MEPs at the grid border in Experiment 1, we increased the size to an 11-by-11 grid (5×5 mm per cell) in Experiments 2–4. The cortical map was assessed by a random order stimulation of all points to prevent any carry-over effect.

The number of TMS pulses delivered during pre/post evaluation differed between experiments: In all experiments, 40 stimuli were applied at 40% MSO prior to the intervention. For the detection of optimal IPI and IDI in Experiments 2–4, 10 paired-pulse stimuli at 110% RMT were applied prior to the intervention at intervals of 1.1, 1.2, 1.3, 1.4, 1.5, 150, 200, 220, 230, and 250 ms, respectively. In addition, 10 stimuli were applied as a test stimulus at 110% RMT. For the MEP stimulus-response curve, 10 stimuli were applied at 90, 100, 120, 130, 140, and 150% RMT, respectively both before and after the intervention (twice after the intervention for Experiments 1 and 2, and once after the intervention for Experiments 3 and 4). For the mapping, pulses were applied at 110% RMT before/after the intervention, i.e., 49/49 (Experiment 1) or 121/121 (Experiments 2–4). For the CSE measurement at 110% RMT, 20 pulses were applied once before and at several time intervals after the intervention, i.e., 20/80 (Experiment 3) and 20/100 (Experiment 4).

MEP analysis

We examined the EMG data, discarding any trials containing muscle pre-activation (rectified pre-stimulus EMG activity above 20 μV). Less than 1% of all trials were rejected due to contamination by muscle activity. Online peak-to-peak MEP amplitudes elicited by doublet stimulation were averaged on a run-to-run basis. Offline peak-to-peak MEP amplitudes were normalized to the baseline to assess CSE changes. For the cortical map, the mean MEP of the full map area was calculated. Data were analyzed using custom written scripts in MATLAB.

Statistical analysis

Statistical analysis was performed using statistical functions in MATLAB (R2017a, The MathWorks, Inc., United States). Datasets undergoing analysis of variance (ANOVA) were assessed for equality of variances using Levene's test, and, if necessary, log-transformed. If significant interactions were detected, post hoc two-tailed *t*-tests were performed using Tukey's test to avoid accumulation of alpha errors. For all statistical analyses, the alpha level was set at $p \leq 0.05$. Results are expressed as mean ± standard error of mean (SEM).

Optimal interpulse interval for SICF and LCD

MEPs elicited by paired pulse TMS were normalized to MEPs elicited by the conditioning stimulus. To investigate optimal intervals for SICF and LCD, an ANOVA with random effect of *subject*, and the fixed factor *interval* was used to assess changes in the dependent variable *normMEP* followed by post-hoc tests (see above).

Online MEP effects during the intervention

To investigate changes during the intervention and between conditions, an ANOVA with random effect of *subject* and fixed factors *run* and *condition* was used to assess the dependent variable *mean MEP of run*.

Offline MEP effects after the intervention

To investigate intervention effects (by comparing pre- and post-intervention MEPs) on the dependent variable *MEP* measured with the stimulus-response curve, we performed an ANOVA for each stimulation intensity with the fixed factors *condition*, *intensity*, and *time*, and the random effect of *subject*. The intervention effects on CSE measured at 110% RMT stimulation were analyzed with an ANOVA on the dependent variable *MEP* with the fixed factors *condition* and *time*, and the random effect of *subject*. Changes in the cortical map were investigated using an ANOVA with the random effect of *subject*, and the fixed factors *condition* and *time* to assess changes in the dependent variable *mean MEP* of the whole map. In case of significant interactions in the ANOVA, post hoc two-tailed t-tests were performed using Tukey's test.

Comparisons of experiments

The conditions that resulted in significant MEP increases were examined with regard to the effect of individualizing the IPI and IDI. Furthermore, conditions of Experiment 1 (without ES) and Experiment 2 (with ES) were examined with regard to the effect of ES. Conditions of Experiment 3 (without ERD triggered stimulation) and Experiment 4 (with ERD triggered stimulation) were examined with regard to the effect of brainstate-dependent ERD-triggered stimulation.

For these analyses, the stimulation intensity of 110% RMT, which was applied before, during and after the intervention was considered. The mean online (during the intervention) and offline (15 min after the intervention) MEP was normalized to the single-pulse TMS MEP before the intervention. This within-subject normalization accounted for sample size differences and subject-dependent biases, e.g., variations in MEP amplitudes between subjects. A randomization test with 1000 repetitions was then applied by shuffling the normalized MEP values of Experiments 1 and 3. A two-sided *t*-test was used to estimate the test statistics at each randomization step. The Monte Carlo P-value was calculated as the proportion of the randomization tests that led to a smaller p-value than the one observed (without randomization).

Comparisons of motor activity during MI and relaxation

The conditions containing MI without ES (due to stimulation artifact) were examined with regard to voluntary activation of the EDC during the MI period.

For this analysis, the root mean square (RMS) of the rectified EMG signal of the MI and the MI_{OFF} period was taken into consideration. A randomization test with 1000 repetitions was then applied by shuffling the RMS values of the MI and the MI_{OFF} period. A two-sided *t*-test was used to estimate the test statistics at each randomization step. The Monte Carlo P-value was calculated as the proportion of the randomization tests that led to a smaller p-value than the one observed (without randomization).

Number of subjects

The number of subjects in each experiment differed due to dropouts (i.e., subjects who did not participate in all conditions of an experiment) and was as follows: Experiment 1: 14 subjects; Experiment 2: 9 subjects; Experiment 3: 15 subjects; Experiment 4: 15 subjects.

One subject participated in Experiments 1–4; one subject participated in Experiments 1–3; three subjects participated in Experiments 1–2; three subjects participated in Experiments 1 and 4; one subject participated in Experiments 2 and 3; one subject participated in Experiments 2 and 4; one subject participated in Experiments 2–4; and three subjects participated in Experiments 3 and 4.

Results

An overview of the significant findings is provided in Table 1.

Experiment 1

Combined MI/DIS increased CSE and enhanced the cortical motor map. This plasticity was associative (with $MI_{ON,}$ but not MI_{OFF} or REST) and persisted beyond the intervention period.

Specifically, ANOVA revealed a significant online effect of *condition* on the mean MEPs of a run (Fig. 2A; condition: $F_{2,911} = 33.2$, $p < 0.001$). Post hoc analysis showed that the mean MEP amplitude during the intervention was significantly higher for MI/DIS than for the other conditions (700.1 ± 23.8 μV; $p < 0.001$; Tukey's test). MI_{OFF}/DIS (473.0 ± 25.6 μV)-and DIS (413.4 ± 22.0 μV) did not differ significantly ($p = 0.950$).

ANOVA revealed a significant effect of *time* in the pre/post motor map and trends for *condition* and *interaction* (Fig. 3; effect of time: $F_{1,105} = 10.4$, $p = 0.002$; effect of condition: $F_{3,105} = 2.2$, $p = 0.090$; effect of interaction: $F_{2,105} = 2.3$, $p = 0.086$).

In addition, a significant offline effect on the MEP amplitude with regard to *time*, *intensity*, *condition* and their *interaction* was observed (Fig. 4; effect of time: $F_{2,11635} = 35.0$, $p < 0.001$; effect of intensity: $F_{6,11635} = 29.3$, $p < 0.001$; effect of condition: $F_{3,11635} = 7.8$, $p < 0.001$; effect of interaction: $F_{36,11635} = 1.8$, $p < 0.001$). Of all the conditions available, MI/DIS showed the highest and most consistent MEP amplitude increases across stimulation intensities after the intervention and at the 60 min follow-up. The MEP amplitudes increased to $241.8 \pm 50.6\%$ of baseline ($p < 0.05$; Tukey's test), particularly at stimulation intensities below the motor threshold. The MI/DIS MEP amplitude at 110% RMT increased significantly post-intervention up to $166.2 \pm 11.1\%$ compared to MI ($130.4 \pm 11.5\%$; $p = 0.034$; Tukey's test), DIS ($127.9 \pm 8.3\%$; $p = 0.019$; Tukey's test), and MI_{OFF}/DIS ($138.6 \pm 9.8\%$; $p = 0.048$; Tukey's test).

Optimal stimulation intervals

In Experiments 2–4, the optimal stimulation intervals that induced maximum SICF and LCD effects, respectively, were determined before the interventions and differed from subject to subject. Interpulse and interdoublet intervals had a significant effect on MEP amplitudes ($F_{10,28} = 37.88$, $p < 0.001$).

In Experiment 2, the maximum SICF occurred at an IPI of 1.1 ms ($n = 1$), 1.2 ms ($n = 3$), 1.3 ms ($n = 3$), 1.4 ms ($n = 1$), or 1.5 ms ($n = 1$). In Experiment 3, the maximum SICF occurred at an IPI of 1.1 ms ($n = 2$), 1.2 ms ($n = 2$), 1.3 ms ($n = 5$), 1.4 ms ($n = 2$), or 1.5 ms ($n = 4$). In Experiment 4, the maximum SICF occurred at an IPI of 1.1 ms ($n = 2$), 1.2 ms ($n = 2$), 1.3 ms ($n = 5$), 1.4 ms ($n = 4$), or 1.5 ms ($n = 2$).

In Experiment 2, the maximum LCD was measured at an IDI of 220 ms ($n = 3$), 230 ms ($n = 5$), or 250 ms ($n = 1$). In Experiment 3, the maximum LCD was measured at an IDI of 200 ms ($n = 4$), 220 ms ($n = 5$), 230 ms ($n = 4$), or 250 ms ($n = 2$). In Experiment 4, the maximum LCD was measured at an IDI of 200 ms ($n = 3$), 220 ms ($n = 6$), 230 ms ($n = 1$), or 250 ms ($n = 5$).

DIS was then delivered at the individually optimized intervals for each subject. Individually adjusted IPI and IDI increased the online effects of MI/DIS on normalized MEP values significantly ($p = 0.007$ in a comparison between Experiments 1 and 3). These effects did not persist after the intervention ($p = 0.366$). However, the after-effects also increased in experiments with individually adjusted IPIs and IDIs (Fig. 7). Immediately after the intervention (see below), MI/DIS revealed a significant increase ($p < 0.001$) that lasted up to 30 min (post 30: $p < 0.001$), but declined thereafter (post 45: $p = 0.991$; post 60: $p = 0.998$ compared to the baseline).

Experiment 2

Concurrent neuromuscular electrical stimulation (ES) to the finger extension muscle targeted by MI/DIS cancelled out the consistent CSE increases across the stimulation intensities observed in Experiment 1 ($p = 0.006$; in a comparison between Experiments 1 and 2). Significant MEP amplitude changes ($p < 0.05$; Tukey's test) occurred for single stimulation intensities and at single time points only (Fig. 5; effect of time: $F_{2,7373} = 8.8$, $p < 0.001$; effect of intensity: $F_{6,7373} = 4.2$, $p < 0.001$; effect of condition: $F_{3,7373} = 1.0$, $p = 0.443$; effect of interaction: $F_{36,7373} = 2.0$, $p < 0.001$).

A comparison of the mean MEPs of the pre/post motor map (effect of time: $F_{2,93} = 0.6$, $p = 0.581$; effect of condition: $F_{3,93} = 0.4$, $p = 0.753$; effect of interaction: $F_{6,93} = 0.7$, $p = 0.683$) using ANOVA revealed no significant effect. Due to the artifacts related to simultaneous ES in all conditions, it was not possible to measure MEP amplitudes *during* the interventions (online effects).

Experiment 3

Expanding this combined MI/DIS intervention to 480 stimuli (instead of 48 stimuli) amplified the sustainability of CSE changes.

Table 1
Overview of experimental conditions that resulted in significant findings. Please note that all experiments have a stimulus-response curve at 15 min, but differ in the follow-up examinations, i.e., with a stimulus-response curve at 60 min (Experiments 1 and 2) or stimulation at 110% RMT at shorter intervals (Experiments 3 and 4). Consistent CSE increases across different stimulation intensities and/or intervals are highlighted in bold. The abbreviation "n.s." stands for non-significant.

Experiment	Main Intervention	Stimulus-response curve (stimulation at % RMT)		CSE at 110% RMT (minutes post intervention)
		15 min	60 min	
1	MI/DIS	**MI/DIS (90, 100, 110, 120)**; **DIS (100, 120, 140)**	**MI/DIS (90, 100, 110, 120, 130)**; MI_{OFF}/DIS (110, 140); DIS (100); MI (120)	–
2	MI/DIS/ES	MI/DIS/ES (110); MI_{OFF}/DIS/ES (110, 120); MI/ES (130)	DIS/ES (120, 140)	–
3	MI/DIS_{10}	**MI/DIS (100, 120, 130, 150)**; **MI/DIS_{10} (100, 110, 140)**; **DIS_{10} (110, 120, 140)**; MI_{10} (110)	–	MI/DIS (15, 30); **MI/DIS_{10} (15, 30, 45, 60)**
4	Triggered $MI/DIS/ES_{10}$	n.s.	–	MI/DIS_{D10} (30)

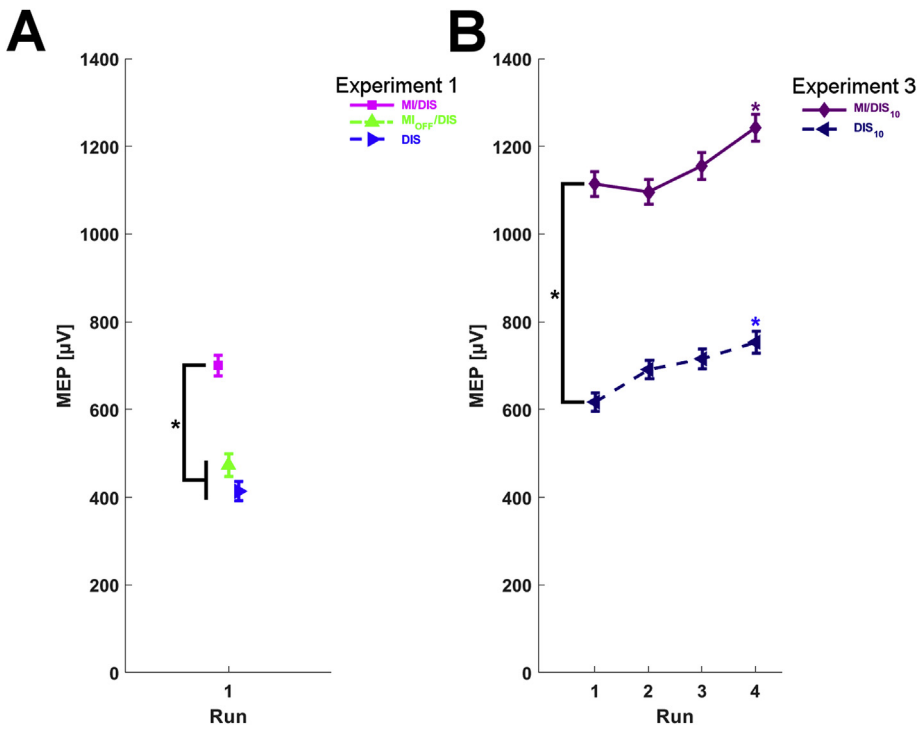

Fig. 2. Time-course of mean MEP amplitude of a run during the intervention. A: In Experiment 1, a run consisted of 6 trials. The condition MI/DIS resulted in a significant increase of the mean MEP amplitude ± SEM (* indicates $p < 0.05$; Tukey's test). B: In Experiment 3, a run consisted of 15 trials. MI/DIS10 resulted in a significant increase of the mean MEP amplitude ± SEM in comparison to DIS10. Moreover, both MI/DIS10 and DIS10 showed a significant increase of the mean MEP of the fourth run in comparison to the first run (* indicates $p < 0.05$; Tukey's test).

Specifically, ANOVA revealed a significant online effect of *condition* on the mean MEPs (Fig. 2B; condition: $F_{2,7439} = 5.67$, $p = 0.016$). The mean MEP amplitude during the intervention was significantly higher for MI/DIS_{10} (1152.1 ± 29.7 µV) than for DIS_{10} (694.2 ± 22.3 µV; $p < 0.001$; Tukey's test). Importantly, both MI/DIS_{10} and DIS_{10} showed a significant increase of the mean MEP amplitude during the intervention ($p < 0.001$; Tukey's test).

Moreover, *time*, *intensity*, and the *interaction* had significant effects on the MEP amplitudes in the input-output curve after the intervention (Fig. 6; effect of time: $F_{1,7680} = 19.19$, $p < 0.001$; effect of intensity: $F_{6,7680} = 9.76$, $p < 0.001$; effect of condition: $F_{3,7680} = 1.15$, $p = 0.327$; effect of interaction: $F_{18,7680} = 3.18$, $p < 0.001$). Individually adjusted IPI and IDI intervals led to the highest and most consistent MEP amplitude increases near motor threshold ($p < 0.05$; Tukey's test). When stimulated at 110% RMT in the follow-up period, both MI/DIS and MI/DIS_{10} showed a significant MEP amplitude increase directly after the intervention (Fig. 7; effect of time: $F_{4,5574} = 22.3$, $p < 0.001$; effect of condition: $F_{3,5574} = 10.1$, $p < 0.001$; effect of interaction: $F_{12,5574} = 6.4$, $p < 0.001$). Immediately after the intervention, MI/DIS revealed the highest increase to 208.4 ± 16.5% of baseline ($p < 0.001$). The MEP amplitude increased significantly in comparison to DIS_{10} (121.8 ± 6.5%; $p < 0.001$), MI/DIS_{10} (158.8 ± 8.5%; $p = 0.008$), and MI_{10} (119.7 ± 7.6%; $p < 0.001$). However, this MI/DIS increase declined during the follow-up period (post 30: $p < 0.001$; post 45: $p = 0.991$; post 60: $p = 0.998$ compared to the baseline). MI/DIS_{10} showed a consistent MEP amplitude increase to an average of 152.6 ± 8.4% of baseline throughout the follow-up period ($p < 0.05$; Tukey's test). MI_{10} showed an increase 45 min after the intervention that was, however, not significant ($p = 0.058$; Tukey's test).

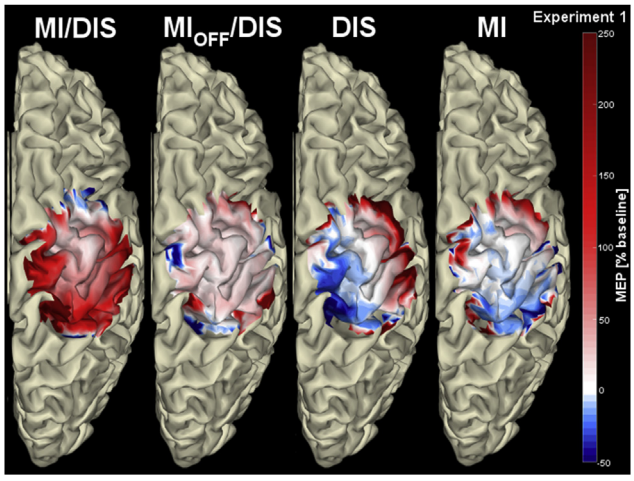

Fig. 3. Changes in pre/post motor map for Experiment 1. The mean MEP of the cortical motor map increased to 203.3 ± 23.8% of baseline in the MI/DIS condition. The interaction of time and condition, however, showed only a trend ($p = 0.086$).

As in Experiment 1, the pre/post motor map showed a significant effect of time but not for condition or interaction (effect of time: $F_{1,119} = 10.1$, $p < 0.01$; effect of condition: $F_{3,119} = 1.1$, $p = 0.365$; effect of interaction: $F_{3,119} = 1.1$, $p = 0.365$).

Experiment 4

The application of MI-related, ERD-triggered stimulation resulted in varying inter-burst intervals and in a mean of 98.6 ± 16.2 MI trials per condition, providing 60 trials in which the predefined ERD threshold was exceeded to trigger the stimulation.

This approach cancelled the plasticity induction observed in the previous experiments ($p = 0.04$; in a comparison between Experiments 3 and 4). No significant increase of the mean MEP amplitude

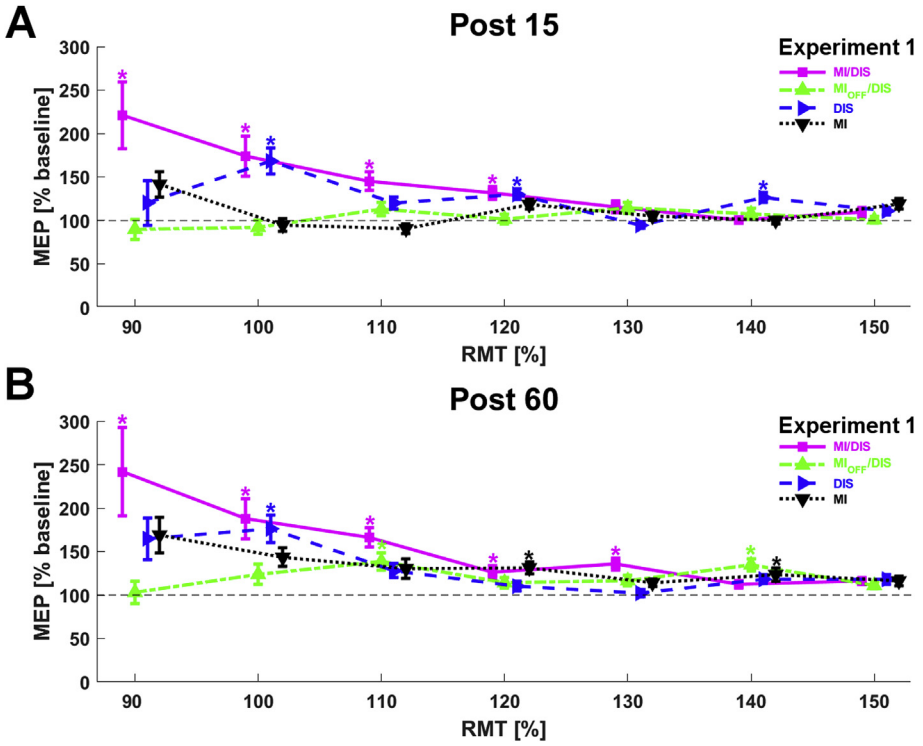

Fig. 4. Input-output changes of MEP amplitude normalized to baseline for Experiment 1. A: Of all the conditions observed, MI/DIS showed the highest and most consistent mean MEP amplitude ± SEM increases across stimulation intensities after the intervention and B for a follow-up of 60 min (* indicates $p < 0.05$, Tukey's test).

was observed *during* the intervention (condition: $F_{3,10805} = 1.84$, $p = 0.185$).

The pre/post motor map showed no significant changes of the mean MEP of the cortical area during any condition (effect of time: $F_{1,149} = 6.1$, $p = 0.015$; effect of condition: $F_{4,149} = 0.5$, $p = 0.760$; effect of interaction: $F_{4,149} = 0.5$, $p = 0.760$).

With regard to the MEP stimulus-response curve, no significant effect of the interaction between *time*, *intensity*, and *condition* on

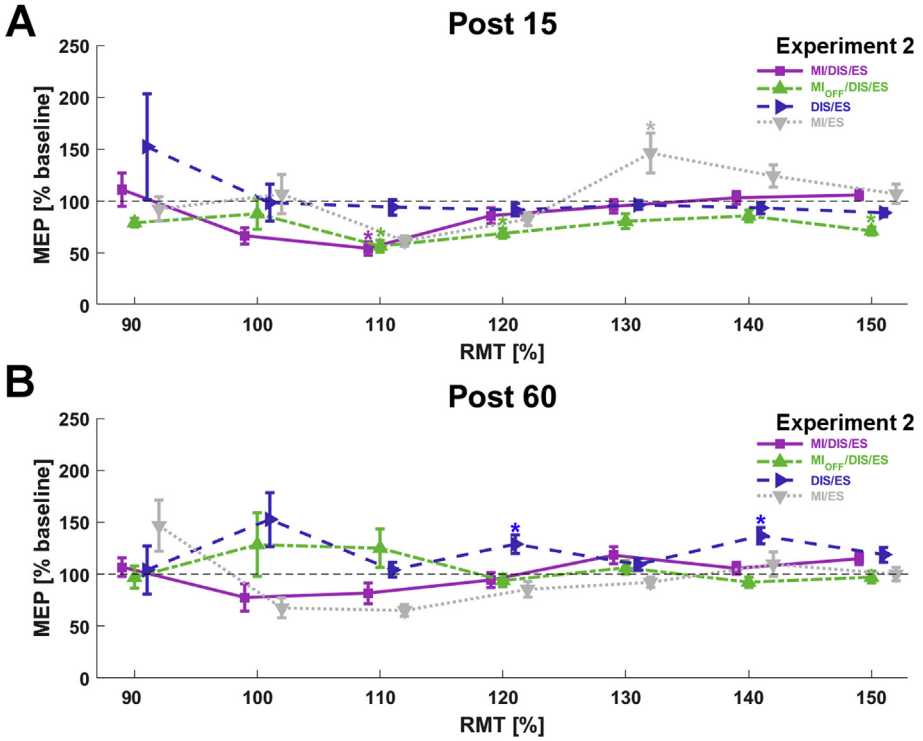

Fig. 5. Input-output changes of MEP amplitude normalized to baseline for Experiment 2. A: Significant mean MEP amplitude ± SEM changes occurred only for single stimulation intensities at single time points after the intervention and B at a follow-up of 60 min (* indicates $p < 0.05$, Tukey's test).

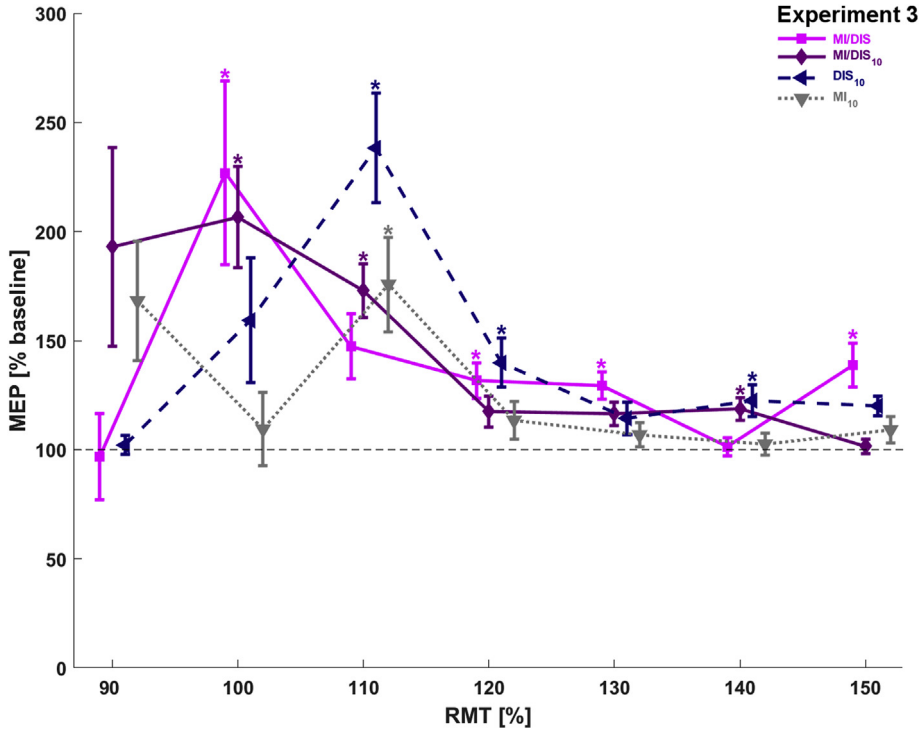

Fig. 6. Input-output changes of MEP amplitude normalized to baseline for Experiment 3. Individually adjusted IPI and IDI intervals led to the highest and most consistent MEP amplitude increases near motor threshold (* indicates $p < 0.05$, Tukey's test).

the MEP amplitude was observed (effect of time: $F_{1,10061} = 26.6$, $p < 0.001$; effect of intensity: $F_{6,10061} = 3.2$, $p < 0.01$; effect of condition: $F_{3,10061} = 2.8$, $p = 0.023$; effect of interaction: $F_{24,10061} = 1.3$, $p = 0.158$). When stimulating at 110% RMT in the follow-up period, MI/DIS_{D10} led to a significant change in MEP amplitude (Fig. 8; effect of time: $F_{5,8904} = 2.6$, $p = 0.034$; effect of condition: $F_{4,8904} = 3.7$, $p = 0.010$; effect of interaction: $F_{20,8904} = 2.3$, $p = 0.002$). However, only at the post 30 min measurement, MI/

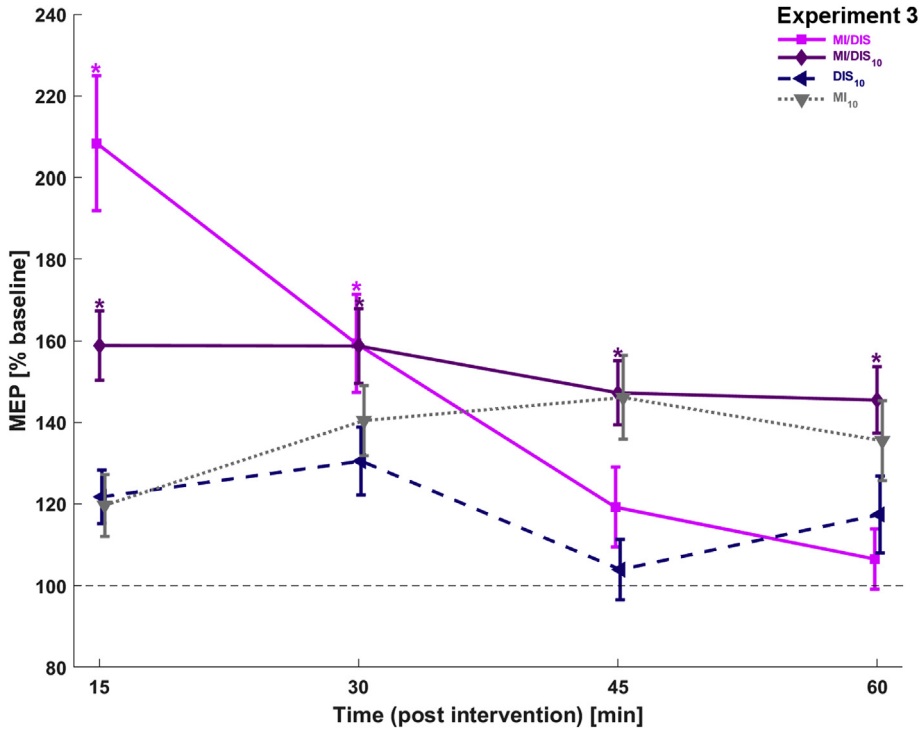

Fig. 7. Time-course of MEP amplitude changes postintervention for Experiment 3. MI/DIS and MI/DIS10 showed a significant mean MEP amplitude ± SEM increase immediately after the intervention, but only MI/DIS10 increased MEP amplitudes significantly up to the follow-up of 60 min (* indicates $p < 0.05$, Tukey's test).

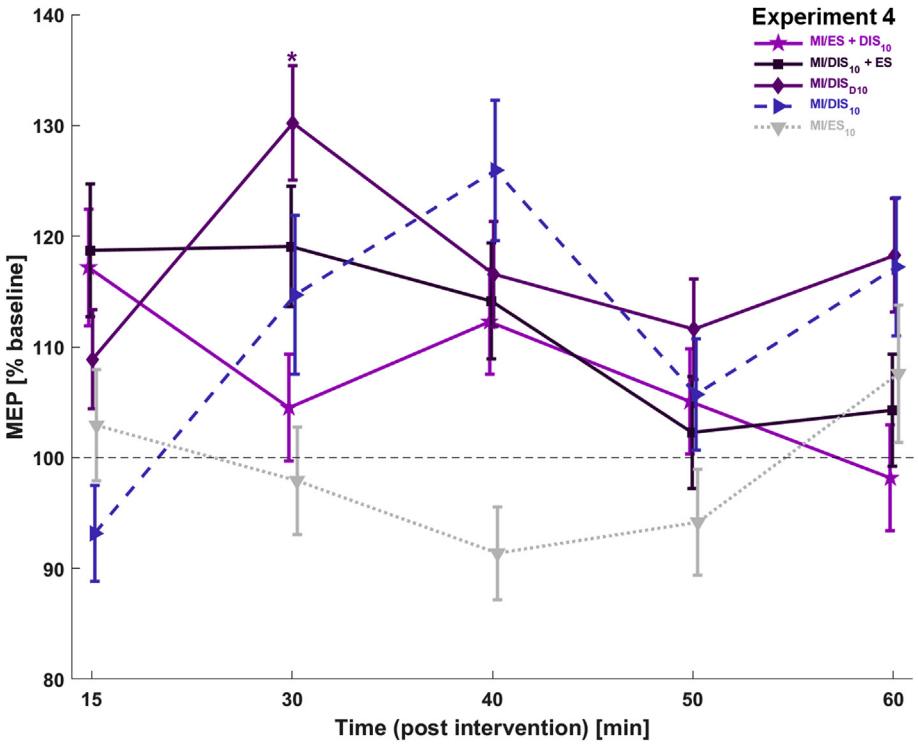

Fig. 8. Time-course of MEP amplitude changes post intervention for Experiment 4. MI/DISD10 led to a significant increase compared to the baseline 30 min after the intervention (* indicates $p < 0.05$, Tukey's test).

DIS_{D10} resulted in a significant increase to $130.2 \pm 5.2\%$ of baseline ($p = 0.004$).

Comparisons of motor activity during MI and relaxation

The RMS of the MI period (3.1 ± 3.6) did not differ significantly from the RMS of the MI_{OFF} period (3.0 ± 3.8; $p = 0.182$).

Discussion

In this study with healthy subjects, we investigated a novel intervention for plasticity induction by combining endogenous and exogenous disinhibition of intracortical inhibitory circuits. MI of finger extension was paired with DIS to the respective cortical motor representation. The combined MI/DIS intervention induced marked and lasting CSE increases across different stimulation intensities. This effect was not observed when each of the interventions was applied alone or when DIS was applied asynchronously to MI, thereby revealing associativity.

Endogenous disinhibition with MI

To induce plasticity, modified PAS protocols [69] have used MI-related brain states such as ERD as the endogenous associative input during cortical [39], peripheral [33,45,70], or combined cortical/peripheral stimulation [41,44,58]. In this context, recent findings indicate that, in addition to postsynaptic $GABA_A$ergic mechanisms [29–31], presynaptic $GABA_B$ergic disinhibition [32] also contributes to the improved excitatory synapse efficiency responsible for the task-specific facilitation of CSE during MI. $GABA_B$ergic disinhibition is therefore a further neurophysiological feature involved in the desynchronization of neural rhythms during both real and imagined movements [32]. With regard to the potential mechanisms of the investigated associative interventions, this implies that the plasticity induction observed could be mediated either by classical pre-post synaptic stimulation [71] or by convergence of two or more presynaptic signals onto a common postsynaptic target, i.e., corticospinal motor neurons in layer V of M1 [72]. Either way, MI amplifies synaptic transmission; its associative pairing with an additional input will thus trigger plasticity via synergistic mechanisms. Changes of corticospinal transmission are functionally relevant in both healthy [73] and injured conditions [74]. Specifically, PAS-induced CSE increases were positively correlated with enhancements in voluntary motor output in both healthy and injured subjects, indicating that there is a connection between the motor output and magnitude of the induced plasticity [74]. However, previous MI-mediated PAS protocols using single TMS pulses led to moderate CSE increases of about 20–30%, i.e., ~120–130% of the pre-intervention baseline, even after intervention periods of ~40–50 min [41,44,45]. The magnitude of plasticity induction therefore needs to be increased to maximize efficacy, while the length of the interventions should be reduced to facilitate their transfer to clinical application.

Exogenous disinhibition with DIS

Previous work on LCD was inconsistent with regard to CSE facilitation during rest [32]. While initial studies demonstrated LCD-mediated facilitation at rest [15,16], recent work observed LCD during MI [32] or voluntary muscle contraction [75], but not at rest [32,75].

Our study complemented this line of research by investigating the induced plasticity for different stimulation intensities and under different conditions. When DIS was applied at rest, the stimulus-response curve at the 60 min follow-up measurement revealed increased CSE as in previous work [11], but for a specific stimulation intensity only, i.e., at 100% RMT (Fig. 4B).

Furthermore, our findings indicate that LCD effects require an endogenous modulation [32,75] to induce plasticity across different stimulation intensities. This is in line with the known task-related modulations of the physiological characteristics of cortical interneuronal populations [76]. All in all, the findings suggest that the combination of DIS and MI promotes LCD and, thereby, lasting LTP effects. Specifically, following a ~90 s intervention, MI/DIS amplified CSE to ~150–200% of baseline.

Disinhibited neural circuitries

Different TMS intensities target distinct neuronal circuitries [77–81] and may provide information about the neural circuitry involved [82]: TMS over the M1 evokes multiple descending volleys, generated by direct (D-wave) and indirect (I-waves) activation of the corticospinal pathway [81]. The stimulation intensity determines the recruitment [80]; intensities below 110% RMT induce MEPs via the recruitment of early I-waves [79], while later I-waves are recruited with increasing stimulation amplitude [77,81]. When the stimulation intensity is increased further, the axons of the corticospinal neurons are directly activated (D-wave) [81]. These earlier findings were based on a monophasic posterior-anterior (PA) current flow. In the present study, however, biphasic pulses with a PA-AP current flow were applied, resulting in a mainly AP-directed current in accordance with the long descending phase of the pulse [83,84]. This produces a more complex pattern of descending volleys than monophasic pulses [84]. The interpretation of differential I-wave recruitment between interventions in this study on the basis of effects at different stimulation intensities therefore remains speculative and requires further investigation.

Our current findings suggest, however, that intracortical circuits may be differently addressed by DIS when comparing unified (Fig. 4) to individualized IPI (Fig. 6), i.e., maximizing CSE increases below vs. near motor threshold, respectively.

When applied to the cortical representation of the EDC at rest, DIS modulated CSE for 60 min after the intervention for low stimulation intensities [79].When paired with MI, however, the amplified CSE was more consistent across different stimulation intensities. Furthermore, the MEP changes measured during the intervention indicate a CSE baseline shift during MI in comparison to MI_{OFF} or REST (Fig. 2). MI appears to modulate the susceptibility of the stimulated intracortical circuits to an excitatory drive similar to a gating mechanism [85].

Modulation of disinhibition

Despite the fact that MI/ES in previous studies enhanced ERD [27] and CSE [28] to a greater extent than MI alone (at least during the intervention), neuromuscular ES did not amplify the effects of the investigated disinhibition protocols on CSE after the intervention in our study. The lack of plasticity induction is, therefore, open to various interpretations: (i) Pairing MI and ES does not generally result in associative plasticity following the intervention. (ii) The intervention dose (6 and 60 trials of MI/ES in Experiments 2 and 4, respectively) was not sufficient to induce plasticity. (iii) Since neuromuscular ES follows an all-or-nothing principle of muscle activation, it cannot be modulated contingently by the level of MI-related ERD like robotic orthoses within brain-machine interfaces (BMI) [53,56,86]. However, previous BMI work of our group indicates that enhancing ERD levels and subsequent motor improvements during associative pairing are critically dependent on the contingency between MI-related ERD and proprioceptive feedback, i.e., when peripheral input occurs *during* MI only [60]. Future work may explore different peripheral stimulation protocols (e.g., lower stimulation intensities or with other modalities such as robot-assisted orthotic movements) to amplify the MI/DIS effects observed in this study.

Our follow-up experiments furthermore revealed that adjusting IPI and IDI for each subject individually may significantly increase the online stimulation effects on CSE in accordance with previous findings [13]. Unlike in previous studies [11,13], these facilatory effects did not manifest themselves for more than 30 min after the intervention. On the basis of the online amplitudes observed (Fig. 2), one would expect to see more pronounced after-effects with individually adjusted IPIs and IDIs (Fig. 7). Possible interpretations remain speculative: Instantaneous excitability and LTP-like plasticity seem to be differently affected by individualized stimulation parameters, thereby suggesting that they are driven by different mechanisms. Alternatively, maximizing instantaneous excitability may subsequently limit lasting LTP in the context of homeostatic meta-plasticity. Another interpretation may be related to the stimulation parameters applied in the non-adjusted intervention (Experiment 1). On the basis of previous findings on optimal stimulation intervals, unified interpulse (IPI; 1.3 ms) and interdoublet intervals (IDI; 220 ms) were applied in all subjects. As intended, these parameters were identical to or at least resemble the intervals identified as optimal for most subjects in the follow-up experiments (individualized IDI ± 10–20 ms). However, previous studies that had shown a significant after-effect of parameter individualization compared the optimal parameters to control conditions with rather distant intervals (individualized IDI ± 50 ms [11]) or excluded subjects from further analysis when the adjusted intervals equaled the predefined ones [13]. This indicates that unified parameters, when preselected adequately, will result in strong CSE increases in the majority of subjects, thereby limiting the additional benefit of individualization.

However, by extending the intervention period, the sustainability of the plasticity could be improved throughout the follow-up period albeit - unlike the short intervention period (Fig. 7) - the maximum CSE did not increase.

Moreover, repetitive pairing of MI and DIS led to more consistent CSE increases across Experiments 1–3 than pairing them on the basis of a predefined ERD threshold (Experiment 4; Fig. 8). Specifically, ERD-triggered stimulation resulted in variable inter-burst intervals and longer periods without stimulation. This might have prevented the build-up of accumulative effects such as those observed in the earlier experiments with consistent inter-burst intervals. This assumption is further supported by studies investigating TBS where the timing between and the duration of the bursts was crucial for facilitation or inhibition of CSE [8,87].

Clinical considerations

In principle, the stimulation paradigm of this study could be applied in several different ways in clinical practice, e.g., for the neurorehabilitation of post-stroke motor function. Stimulation-induced after-effects might be used to prime subsequent therapy [88], e.g., intervention-related LTD-induction with ensuing physiotherapy [89]. Such an approach would, however, be limited to patients with residual motor function who are capable of participating in movement exercises. However, if active movements are no longer possible, alternative approaches are necessary to achieve lasting and clinically relevant after-effects. Moreover, the suggested disinhibition protocols seem to be better suited to LTP-induction. In this context, we propose that the DIS/MI protocol introduced here could actually serve as a therapeutic intervention to enhance movement relevant motor networks, a hypothesis that needs to be confirmed in future studies. Along these lines, DIS/MI may be combined with concurrent proprioceptive feedback [60] via a robotic orthosis that moves the paralyzed hand/arm [63,64,86,90].

Such brain-machine interfaces would provide state-dependent [91], paired associative stimulation (to cortex and periphery) which has been shown to recruit additional corticospinal pathways [44,58,92].

Limitations and future perspectives

Future studies need to investigate the sustainability of MI/DIS plasticity induction for longer follow-up periods, particularly since previous work indicates that CSE increases of DIS without MI last no longer than 60 min [11]. Furthermore, the functional relevance of our physiological findings should be tested with regard to behavioral outcome parameters on the basis of previous protocols. The latter indicate that there is a correlation between the magnitude of induced plasticity and the voluntary motor output in both injured and healthy subjects [73,74].

Future studies may also include an additional control condition. Since stimulation induced larger MEPs with MI than without, the stronger after-effects might also be caused by stronger stimulation-induced muscle twitches. An intervention (without MI) using a stimulus intensity that produces MEPs of similar amplitude to the stimulation with MI is desirable to exclude a contribution of larger MEP amplitudes (and a stronger afferent input caused by the stimulation-evoked muscle twitch). In any case, the present findings indicate that the combined MI/DIS intervention is superior to each of the approaches applied independently for inducing marked and lasting motor cortex plasticity within a remarkably short period of time. This may lead to new interventions for pathological conditions, e.g., post-stroke paralysis, where no active movement is possible independent of the underlying neurophysiological mechanism of the observed effects.

For individualizing the IPI, we investigated a range from 1.1 to 1.5 ms, detected significant increases in every subject, and established 1.3 ms as the mean optimal value similar to previous studies [11]. However, the first peak of the SICF interaction, reflecting the first I-wave, might also occur later. To capture the peak-IPI in every participant, future studies should consider extending the range investigated beyond 1.5 ms.

In the follow-up experiments, we modified the paradigm by individualizing IPI and IDI intervals, increasing the grid size of the examined cortical motor map, changing the intervals to capture lasting changes of CSE, and modifying the timing between DIS and ES. These modifications were conducted on the basis of findings in the previous experiments. While the aim of this pragmatic approach was to maximize CSE increases and to efficiently explore the impact of modulating factors, this may have limited rigorous comparisons between the different experiments. However, since each of the follow-up experiments was designed with sufficient control conditions and included the main MI/DIS intervention, the respective results may provide insight even independently of the first experiment. Nonetheless, future studies may pose more specific questions on the basis of the findings presented here and then apply more rigorous study designs. The negative findings with regard to the cortical motor maps in the follow-up experiments may, for example, be explained by the larger grid size applied in these later measurements. Specifically, the inclusion of (potentially non-responsive) stimulation points beyond the border zone of the previous map might have obscured positive responses in the center of the grid when calculating the mean MEP of the motor map.

In conclusion, the combination of endogenous and exogenous disinhibition of intracortical circuits for a remarkably short period of time augments lasting plasticity induction in the human motor cortex. This intervention may thus provide a therapeutic backdoor when active movements are no longer possible, e.g., for hand paralysis after stroke.

Authors'Contributions

L.Z. and A.G. designed research; L.Z. and R.S. performed research; L.Z., R.S., and A.G. analyzed data; L.Z. and A.G. wrote the manuscript.

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Acknowledgments

Conflict of Interest: None declared.

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