Disinhibition of intracortical networks to augment crossed and uncrossed corticospinal pathways

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Table of Abbreviations

Alpha motoneuron	αMNs
Biceps brachii	BB
Brain-machine interface	BMI
Corticospinal excitability	CSE
Corticospinal tract	CST
Corticospinal tract	CST
Disinhibition stimulation	DIS
Electrical stimulation	ES
Event-related desynchronization	ERD
Extensor digitorum communis	EDC
Fugl-Meyer	FM
Gamma-aminobutyric acid	GABA
Inter-doublet interval	IDI
Interhemispheric inhibition	IHI
Interpulse interval	IPI
Intracortical inhibition	ICI
Late cortical disinhibition	LCD
Long intracortical inhibition	LICI
Long-term potentiation	LTP
Modified Ashworth spasticity	MAS
Motor evoked potential	MEP
Motor imagery	MI
Motor-evoked potential	MEP
N-methyl-D-aspartate	NMDA
Paired associative stimulation	PAS
Primary motor cortex	M1
Repetitive paired TMS at I-wave periodicity	ITMS
Repetitive transcranial magnetic stimulation	rTMS
Short intracortical facilitation	SICF

Short intracortical inhibition	SICI
Theta burst stimulation	TBS
Transcranial magnetic stimulation	TMS

1 Abstract

Multi-modal neurorehabilitation models for stroke patients recommend an approach based on severity of hemisphere damage. If the ipsilesional primary motor cortex (M1) is still intact, the crossed corticospinal tract (CST) can be targeted. However, severely affected patients rely on contralesional motor cortices and therefore the uncrossed CST.

Disinhibition of intracortical networks can facilitate plasticity induction in the CST and therefore recovery. Motor execution or imagery (MI) results in an endogenous disinhibition. Exogenous modulation of inhibitory intracortical networks is achieved by a repetitive paired-pulse transcranial magnetic stimulation (TMS) protocol, referred to as DIS, or electrical stimulation (ES). A combination of the different disinhibition protocols has not been tested yet. Furthermore, the efficacy of targeting the uncrossed CST from contralesional M1 remains unclear. As the presence of ipsilateral motor evoked potentials (MEPs) from the uncrossed CST in hand muscles has escaped recognition, limited data is available.

We hypothesized that lasting corticospinal excitability (CSE) changes could be achieved by associatively pairing endogenous modulation with exogenous stimulation of the same intracortical circuits. Furthermore, we investigated in detail the uncrossed CST.

In this line of work, we combined MI of finger extension with DIS to modulate uncrossed CST in healthy subjects. For uncrossed CST, we tested different stimulation protocols for optimal detection of MEPs and combined DIS with active motor execution for CSE enhancement. Furthermore, modulation of ipsilateral CST was investigated in healthy subjects and severely affected stroke patients.

MI combined with DIS resulted in a significant and persistent increase of contralateral CSE, e.g. of the crossed CST. A longer intervention duration further enhanced sustainability of CSE changes. MI alone, DIS alone, or MI/DIS in combination with ES did not result in changes of CSE.

Ipsilateral MEPs from the uncrossed CST were reliably measured after TMS during biceps brachii (BB) contraction with a coil orientation of 45° to the sagittal line. Furthermore, paired-pulse TMS facilitated ipsilateral MEPs. DIS alone, but not in combination with MI, resulted in plasticity induction of ipsilateral CST. Additionally, DIS in combination with motor execution resulted in CSE increases in both healthy subjects and severely affected stroke patients.

Taken together, we designed and improved two effective associative stimulation protocols combining endogenous and exogenous disinhibition of intracortical circuits. Each protocol was optimized to augment plasticity induction in contralateral or ipsilateral CSE, respectively. Both represent new, efficient interventions targeting either crossed or uncrossed CST and can be applied according to intactness of ipsilesional CST.

This thesis may help in developing new therapeutic approaches in stroke rehabilitation, especially for severely affected stroke patients with no residual control of their paretic hand.

2 Introduction

2.1 Stroke

In adults, stroke is the second most common cause of death (Feigin et al., 2009; Mozaffarian et al., 2015) and the global burden of stroke continues to grow (Feigin et al., 2015). Although most stroke survivors achieve at least some spontaneous recovery, it remains the leading cause of chronic disability in the developed world (Veerbeek et al., 2011; Go et al., 2013). About 70–75% of chronic stroke survivors suffer from permanent disability after the ictus even after participation in rehabilitative training programs (Feigin et al., 2009; Mozaffarian et al., 2015). Motor dysfunction, especially hemiparesis, the simultaneous weakness of an arm and leg on one side, is the most frequent consequence of stroke (Rathore et al., 2002). The reduced arm function is especially disadvantageous to quality of life (Nichols-Larsen et al., 2005). Apart from motor weakness, patients with moderate-to-severe hemiparesis suffer from motor deficits associated with flexor hypertonia and degradation of selective muscle activation (Uehara et al., 2015). To counteract the flexor hypertonia, it is important to induce an extensor-specific long-term potentiation (LTP)-like plasticity. Collectively, stroke causes significant human suffering and poses a major economic burden on the society.

Most stroke survivors experience some degree of functional recovery (Veerbeek et al., 2011; Go et al., 2013). Stroke recovery is divided into acute, post-acute, and chronic stages. The acute stage is commonly defined as the first one to four weeks after stroke, corresponding to an acute hospital setting, although this varies greatly from country to country. In the acute phase, there is a period of enhanced spontaneous plasticity occurring one to four weeks after stroke, which can support some restoration of function (Butefisch et al., 2003; Murphy and Corbett, 2009; Ward, 2017). The post-acute stage is defined as the period immediately after discharge from the intensive care unit. During this stage, recovery of learning-dependent processes occur over longer time frames before plateauing around six months after stroke (Langhorne et al., 2011). The chronic period starts six months after stroke onset. This stage is characterized by a small probability of significant improvement thereafter (Langhorne et al., 2011).

Extent and timeline of stroke recovery vary depending on factors such as size and location of the infarction (Plow et al., 2016). In patients with a functional ipsilesional corticospinal tract (CST), initial motor recovery of the upper-extremity impairment occurs with a proportional resolution of 70% of lost function within the first three months (Byblow et al., 2015; Winters et al., 2015). This proportional recovery is attributed to spontaneous neurological recovery and is thought to be largely independent of any rehabilitation (Byblow et al., 2015; Winters et al., 2015). However, in patients with severe stroke, recovery is proportional to initial severity in about half of the patients, with the other half making no recovery at all (Byblow et al., 2015; Winters et al., 2015). Thus, severity of the initial deficit after stroke is the predominant predictor of recovery (Prabhakaran et al., 2008; Ward, 2017). To increase resolution of impairment above 70% and achieve long-lasting, clinically exploitable effects even in severely affected stroke patients, novel therapeutic models and their investigation are required.

Motor recovery after stroke is due to massive neuronal reorganization occurring both locally and remotely to the lesion site (Cramer, 2008; Murphy and Corbett, 2009). Functional restoration after stroke is an ongoing challenge and models taking into account the extent of structural damage and availability of residual motor pathways

have been proposed (Bradnam et al., 2013b; Di Pino et al., 2014a; Plow et al., 2016). Less affected patients rely on the ipsilesional primary motor cortex (M1), while more affected patients rely on undamaged contralesional motor cortices for recovery (Bradnam et al., 2013b; Di Pino et al., 2014a; Plow et al., 2016). This is because less affected patients have a higher structural reserve and crossed CST of ipsilesional M1 is still functional (Di Lazzaro et al., 2008; Halko et al., 2011; Sung et al., 2013). In these patients, abnormal over-excitation of contralesional M1 inhibits corticospinal excitability (CSE) of ipsilesional M1 via interhemispheric inhibition (IHI) (Murase et al., 2004). Thus, facilitation of ipsilesional M1 in combination with concurrent inhibition of contralesional M1 is the most promising approach towards recovery (Di Lazzaro et al., 2008; Halko et al., 2011; Sung et al., 2013). However, this may be ineffective in patients who suffered from extensive damage to the crossed CST (Bradnam et al., 2013b; Di Pino et al., 2014a; Plow et al., 2016). In these patients, contralesional areas have to be recruited to take over lost function (Nowak et al., 2009; Riecker et al., 2010; Small et al., 2013; Grefkes and Ward, 2014; Sankarasubramanian et al., 2017; McCambridge et al., 2018).

Based on the premise that severity of the initial deficit after stroke is important for therapeutic approach, promoting ipsilesional M1 activity is the optimal approach towards neurorehabilitation for less affected patients (Plow et al., 2016). In contrast, in severely affected patients, facilitation of contralesional M1 might be the preferred neurorehabilitation approach (Plow et al., 2016).

2.2 Corticospinal tract

The common target of plasticity-inducing protocols is the CST. CST originates from a wide variety of cortical areas, each with different functions, e.g., M1, the dorsal and ventral premotor cortices, supplementary motor area, and cingulate motor areas (Dum and Strick, 2005). M1 has direct and indirect projections to alpha motoneurons (αMNs) in the spinal cord (Brinkman and Kuypers, 1973). The direct CST descends in the internal capsule to the brainstem and onto the spinal cord terminating onto αMNs in the ventral horn (Fries et al., 1993). The indirect pathways descend alongside reticulospinal, rubrospinal, and tectospinal tracts (Lemon, 2008).

Although the classical view of the CST is that of a crossed pathway, uncrossed ipsilateral projections exist and contribute to motor execution (Verstynen et al., 2005; Uehara et al., 2011a; van den Berg et al., 2011; Uehara et al., 2013). The pathways mediating ipsilateral responses and their relationship to contralateral corticospinal projections have not been fully defined. A small proportion of the global population of pyramidal axons (8 to 10%) contributes to a monosynaptic corticospinal pathway (Gerloff et al., 1998), which projects mainly to spinal motor neurons innervating truncal and proximal upper limb muscles (Brinkman and Kuypers, 1973). Interhemispheric connections such as the corpus callosum projecting to the contralateral motor cortex might also drive ipsilateral CST (Brus-Ramer et al., 2009). Furthermore, oligosynaptic pathways may contribute to ipsilateral CST, e.g., the reticulospinal pathway. The majority of reticulospinal projections are ipsilateral (Rho et al., 1997) and primate studies showed that their stimulation resulted in the recruitment of ipsilateral shoulder muscles (Herbert et al., 2015). Another candidate system is the corticospinal pathway crossing twice, once at the level of the pyramidal decussation and once more at the level of the spinal cord (Gerloff et al., 1998; Wahl et al., 2017). Furthermore, there is evidence for the involvement of propriospinal projections (Mazevet et al., 2003; Stinear and Byblow, 2004b).

2.3 Disinhibition

2.3.1 Excitation inhibition balance

Modulation of CST and thereby motor recovery can be facilitated by a functional decrease of gamma-aminobutyric acid (GABA)-ergic inhibition (Clarkson et al., 2010; Lazar et al., 2010; Blicher et al., 2015). Simultaneously, plasticity and release of brain-derived neurotrophic factor can be promoted by changes of glutamatergic properties (Citri and Malenka, 2008; Carmichael, 2012). This results in cortical LTP being mediated by N-methyl-D-aspartate (NMDA) receptor activation (Tsumoto, 1992; Hess et al., 1996). The NMDA receptor is characterized by a voltage-sensitive magnesium block (Moriyoshi et al., 1991). Disinhibition removes this magnesium block, resulting in stronger NMDA receptor-mediated postsynaptic responses by virtue of increased intracellular calcium entry, which in turn provides a gating mechanism for LTP (Ziemann and Siebner, 2008).

Therefore, consideration of intracortical networks and their interactions are relevant for the design of LTP-like plasticity-inducing interventions. The interactions between GABAergic interneurons and glutamatergic principal cells are reciprocal, e.g., interneurons inhibit principal cells and are excited by them. Intracortical properties can be probed with paired-pulse transcranial magnetic stimulation (TMS). If a subthreshold conditioning stimulus is applied at very short interstimulus intervals of 1–5ms, a short intracortical inhibition (SICI) is detected (Kujirai et al., 1993). However, if the conditioning stimulus is suprathreshold, a short intracortical facilitation (SICF) is triggered (Tokimura et al., 1996). Another activation of inhibitory networks is observed when a suprathreshold conditioning stimulus is applied 50–200ms prior to test stimulus onset, e.g., long intracortical inhibition (LICI) (Valls-Solé et al., 1992). This postsynaptic inhibition (McDonnell et al., 2006) is followed by a presynaptic disinhibition resulting in a late cortical disinhibition (LCD) (Cash et al., 2010). Different receptors mediate the inhibitory and facilitatory drive. Whereas SICI is driven by $GABA_A$ receptors (Ziemann et al., 1996a, 2015), LICI and LCD are mediated by $GABA_B$ receptors (McDonnell et al., 2006; Cash et al., 2010). Intracortical facilitation on the other hand is glutamatergic (Liepert et al., 1997).

Taken together, the intracortical networks play a crucial role in modulating cortical motor output, a process that depends on the balance between the activity of excitatory and inhibitory circuits.

2.3.2 Transcranial magnetic stimulation

Evidence has accumulated demonstrating that TMS provides a valuable tool for interventional neurophysiology applications (Groppa et al., 2012) and exogenous disinhibition of intracortical networks (Cash et al., 2016). TMS modulates brain activity in a specific, distributed, cortico-subcortical network and consequently induces controlled and controllable manipulations in behavior (Rossi et al., 2009). Different TMS protocols have been developed for the induction of plasticity, e.g., repetitive TMS (rTMS) (Fitzgerald et al., 2006), theta burst stimulation (TBS) (Huang et al., 2005), and paired associative stimulation (PAS) (Stefan et al., 2000), to name a few (for an overview, see (Ziemann et al., 2008)). Furthermore, paired-pulse TMS enables not only the assessment of intra-cortical excitatory and inhibitory circuits, but also their modulation.

Through targeting of SICF by paired-pulse TMS, glutamatergic intracortical excitatory interneurons can be activated (Tokimura et al., 1996). The activation is due

to interaction and summation of different I-waves at corticospinal neurons (Ziemann and Rothwell, 2000; Ilić et al., 2002) resulting in an increased firing probability (Ziemann et al., 1998). Based on these observations, Thickbroom et al., 2006, designed a protocol to target synaptic events by repetitive paired TMS at I-wave periodicity (ITMS). ITMS targets facilitatory I-wave networks using paired TMS stimuli of equal strength with an interpulse interval (IPI) corresponding to the periodicity of I-waves (~1.5ms) delivered for several minutes at a rate of 0.2Hz (Thickbroom et al., 2006). The online effect during the ITMS intervention is a steady increase of paired-pulse motor-evoked potential (MEP) (Di Lazzaro et al., 2007). The offline effect of ITMS is a consistent increase of CSE (Di Lazzaro et al., 2007). Furthermore, on the one hand, doublets targeting SICF induce disinhibition (Cash et al., 2010, 2011), but on the other hand, magnitude and site of I-wave interaction by SICF can be modulated by disinhibition (Cash et al., 2011). In detail, SICF is enhanced during LCD and LCD is observed during SICF, resulting in the hypothesis that disinhibition mediated through paired-pulse TMS improves excitatory synapse efficiency to achieve an overall increase in corticomotor excitability (Cash et al., 2011, 2016).

On the whole, disinhibition may have a gating role for LTP (Ziemann and Siebner, 2008), and TMS interventions targeting LTP could be more effective if delivered during disinhibition evoked by a priming pulse (Cash et al., 2011). Therefore, a novel approach was suggested that uses an ITMS train to trigger the disinhibition exogenously by the application of a suitably timed priming stimulus and thereby incorporating LCD within repetitive ITMS (Cash et al., 2016). In detail, a series of trains consisting of four ITMS doublets was delivered such that each doublet in the train (after the first) was delivered during the LCD evoked by its preceding doublet (Cash et al., 2016). Cash et al. (2016) improved via their novel disinhibition protocol, termed DIS, the efficacy of LTP-like plasticity induction of ITMS by targeting disinhibition, and they were able to shorten the time of intervention to approximately 1.5min while still containing a high topographical specificity.

Taken together, SICF reflects activation of glutamatergic excitatory cortical interneurons responsible for I-wave generation (Tokimura et al., 1996; Ziemann and Rothwell, 2000; Ilić et al., 2002). LCD likely reflects $GABA_B$ergic disinhibition (Cash et al., 2011). The combination of both modulates the CSE in a synergistic way and induces LTP-like plasticity (Cash et al., 2016).

2.3.3 Motor execution and imagery

Intracortical networks can be endogenously modulated via active motor execution or motor imagery (MI).

During unilateral hand movement, CSE of both motor cortices is modulated (Liepert et al., 2001; Hortobágyi et al., 2003; Zijdewind et al., 2006; Stippich et al., 2007; Perez and Cohen, 2008a; Chiou et al., 2013). This modulation is accompanied by a decrease of intracortical inhibition (ICI) (Goodwill et al., 2012; Chiou et al., 2013) driven by GABAergic modulation (Schneider et al., 2002). Furthermore, during voluntary contraction, I-wave generating neurons are facilitated (Di Lazzaro et al., 2004). In detail, activating the contralateral side may induce a decrease of IHI from the opposite cortex (Liang et al., 2011) and disinhibit the intracortical circuits (Muellbacher et al., 2000; Perez and Cohen, 2008a), resulting in a facilitation of CSE.

Regarding stroke rehabilitation, one has to also consider the fact that some patients are suffering from severe paresis and therefore MI represents an alternative

approach towards disinhibition in comparison to active motor execution. MI may be considered the mental rehearsal of a movement without any overt movement, engaging a variety of supraspinal structures without resulting in any outflow from the spinal motor neuron pool. Like actual motor execution, MI engages motor cortical areas and other associated areas (Gerardin et al., 2000; Porro et al., 2000). Furthermore, MI also results in a sensorimotor event-related desynchronization (ERD) (Pfurtscheller and Neuper, 1997; Lotze et al., 1999; Neuper et al., 2005; Miller et al., 2007, 2010; Kaiser et al., 2011). Specifically, kinesthetic MI, which involves imagining the feeling produced by actual task performance (Hall et al., 1985), induces a spatial and temporal modulation of motor cortical function that mirrors the modulation observed during actual motor practice (Stinear et al., 2006). MI-related ERD enhances cortical pyramidal neuron excitability through a disinhibition of I-wave generating neurons and a significant enhancement of cortical pyramidal neuron excitability (Takemi et al., 2013; Chong and Stinear, 2017). This disinhibition leads to reduced SICI, i.e., downregulation of $GABA_A$ergic activity (Abbruzzese et al., 1999; Stinear and Byblow, 2004a; Takemi et al., 2013). Furthermore, MI facilitates LCD, corresponding to recruitment of presynaptic $GABA_B$ disinhibition that is not specific to muscles (Chong and Stinear, 2017). Overall, MI represents a potential technique for modulation of intracortical properties for people with neurological impairments, such as stroke, who cannot perform an active motor execution of the paretic limb.

2.3.4 Electrical stimulation

Passive movements induce patterns in the electromagnetic field generated by the brain similar to those observed during motor execution or motor imagery (Müller et al., 2003; Müller-Putz et al., 2007). Neuromuscular electrical stimulation (ES) in patients with motor function impairment in the upper extremities has been employed as a rehabilitative treatment option (Kimberley et al., 2004; Ring and Rosenthal, 2005; Thorsen et al., 2013; Quandt and Hummel, 2014; Stein et al., 2015). During ES, bursts of short, coordinated pulses of electrical charge generate an electrical field that triggers action potentials in afferent and efferent neural pathways. The efferent neural pathways, in return, facilitate functional movement in weak muscles (Pomeroy et al., 2006). In parallel, activity triggered in afferent pathways carries action potentials to the spinal cord where various reflexes are generated, e.g., cross-extension reflex and the flexion reflex (Quandt and Hummel, 2014; Stein et al., 2015). Additionally, interneurons are activated and transmit signals that eventually reach the cortex (Quandt and Hummel, 2014; Stein et al., 2015). Via these signals, ES is able to modulate IHI similar to unilateral motor training and mirror visual feedback training (Swayne et al., 2006; Avanzino et al., 2014b, 2014a). In detail, ES changes the excitability of the transcallosal pathway and thereby increases IHI (Gueugneau et al., 2017). Several studies using TMS have suggested that ES can change corticospinal excitability (Mang et al., 2010; Bergquist et al., 2011; Chipchase et al., 2011; Miyata and Usuda, 2015). A positive impact has been observed on the contralesional postcentral gyrus (Kimberley et al., 2004; Page et al., 2010), e.g., enhanced cortical activation. However, changes in the ipsilesional hemisphere correlated with reduced cortical activation (Wei et al., 2013). These observations are in concordance with bilateral models, i.e., one can observe a trend towards severe impairment leading to activation of the contralesional site, whereas less impaired patients tend to recruit the ipsilesional site (Quandt and Hummel, 2014). Furthermore, ES enhanced ERD (Reynolds et al., 2015) and CSE (Kaneko et al., 2014) during MI.

2.4 Aim of doctoral thesis

Taken together, an enhanced plasticity induction is possible during disinhibition of intracortical networks.

Therefore, we investigated if the combination of different protocols targeting disinhibition acts in a synergistic way to further enhance the neuroplasticity inducing effects of a single method approach. In detail, we hypothesized that combining exogenous inhibition of $GABA_B$ receptor activity by TMS or of IHI by ES with endogenous inhibition of $GABA_A$ and $GABA_B$ receptor activity by motor execution or MI enhances SICF, and that this state mediates an increase in excitatory synaptic efficacy. We tested various combinations in healthy subjects investigating the contralateral CST.

Because facilitation of contralateral CST and thereby the ipsilesional hemisphere may be insufficient or even erroneous as a therapeutic approach in severely affected patients, we examined next the uncrossed ipsilateral pathways as the preferred neurorehabilitation target.

First, we explored via different stimulation protocols if ipsilateral pathways can be detected in the wrist and finger extensor muscle of healthy subjects, a muscle not examined in detail by previous studies. Specifically, we investigated in detail the optimal stimulation protocol to elicit reliable ipsilateral MEPs and whether the ipsilateral CST underlies an I-wave generating neuronal network.

Finally, we assessed the potential of the combined disinhibition protocols to facilitate ipsilateral uncrossed CST. This investigation was undertaken in both healthy subjects and chronic, severely affected stroke patients.

All presented studies are in a similar experimental setup for comparability. Overall, disinhibition protocols were designed with the hypothesis of inducing plastic changes that could be translated into novel therapeutic applications for rehabilitation of severely affected stroke patients with no voluntary control of their muscle activity.

3 Included publications

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

Plasticity induction is considerably influenced by the balance between GABAergic inhibition and glutamatergic excitation within intracortical circuits (Benali et al., 2008). Therefore, different disinhibition protocols were combined in order to further enhance their individual plasticity inducing effects on CSE. Disinhibition can shift this balance away from inhibition (Hensch and Fagiolini, 2005) and has been shown to facilitate neuroplasticity, e.g., LTP of CSE. Accordingly, we chose a protocol targeting exogenous disinhibition via a paired-pulse rTMS protocol termed DIS (Cash et al., 2016). This protocol combines the inhibition of $GABA_B$ergic mediated inhibition and glutamatergic mediated facilitation (Thickbroom et al., 2006; Cash et al., 2016). As the protocol was so far only applied during rest, we combined DIS with kinesthetic MI. We chose MI as it has been shown to lead to a disinhibition via modulation of $GABA_A$ergic (Takemi et al., 2013) and $GABA_B$ergic (Chong and Stinear, 2017) receptors. Furthermore, because we designed our study with a translational approach for severely affected stroke patients, MI is a potential substitute for motor execution.

To test for the optimal combinations of disinhibition protocols, four experiments were designed. The main aim of all experiments was to investigate modulating factors and enhance CSE increases. The first experiment consisted of a combination of MI and DIS, i.e., DIS was applied either during MI, after MI, or at rest. Furthermore, MI alone was tested. The three follow-up experiments were designed on the basis of the findings of the previous experiments. The second experiment investigated the addition of ES, a feedback known to enhance MI-related ERD (Reynolds et al., 2015) and to increase CSE (Kaneko et al., 2014). In the third experiment, we adjusted the duration of intervention, as intervention duration of paired-pulse rTMS is known to influence CSE (Murray et al., 2011). For the fourth experiment, a closed-loop stimulation paradigm was designed in order to further enhance CSE. This adjustment was performed based on the premise that single-pulse TMS stimulation during an increased ERD, and not in a random order, resulted in enhanced CSE (Kraus et al., 2016a) and to ensure that each subject received the same constant task-related demand. Furthermore, ES was applied at different intervals in relation to DIS in order to investigate paired associative stimulation.

Online effects during intervention were tested via the analysis of paired-pulse MEP. Offline effects on CSE were analyzed via a stimulus-response curve, a cortical map, and/or test stimuli at 110% resting motor threshold (RMT).

The combination of the two disinhibition protocols, e.g., MI and DIS, resulted in a strong CSE increase. The increase was detected across different stimulation intensities and lasted for the total duration of measurement, e.g., 60min. The LTP-like plasticity induction was not observed when each of the disinhibition protocols was applied alone, when DIS was applied asynchronously to MI, or when DIS and MI were combined with ES. Furthermore, the closed-loop ERD-triggered approach did not result in significant changes. During the intervention, the combined MI/DIS protocol resulted in significantly higher CSE. Longer stimulation duration resulted in a significant increase of the mean MEP amplitude in the course of the intervention. Furthermore, by expanding duration of MI/DIS intervention, the persistence of CSE changes was amplified. No significant increase of the mean MEP amplitude was observed during the closed-loop approach. No significant changes were found in the pre/post motor map. Individual adjustment of IPI and inter-doublet interval (IDI) increased the online effects of MI/DIS, but not the offline effects.

Taken together, the combination of two protocols targeting disinhibition, e.g., MI and DIS, acted in a synergistic way to further enhance the neuroplasticity inducing effects.

3.2 Detection and enhancement of ipsilateral connections in the human brain

As ipsilateral M1 has some degree of control over muscles of ipsilateral limbs (Uehara et al., 2011b; Bradnam et al., 2013b; Tazoe and Perez, 2014) a more detailed understanding of ipsilateral CSE is required. TMS can be used to study ipsilateral CSE if prerequisites are met, e.g., muscle preactivation and high TMS intensities (Ziemann et al., 1999; Tazoe and Perez, 2014; McCambridge et al., 2016). As multiple factors influence ipsilateral CSE and to this date, insufficient data is available about intracortical properties modulating ipsilateral CSE, the purpose of this study was to measure consistent and reliable responses. Furthermore, we analyzed the underlying neuronal physiology, e.g., intracortical connections. We first investigated the optimal stimulation protocol for eliciting ipsilateral MEPs. Furthermore, after refining detection of ipsilateral CSE and confirming interneuronal properties of ipsilateral CST favoring

DIS, we consequently analyzed plasticity inducing efficiency of disinhibition protocols, namely DIS and MI. We hypothesized that MI led to a general release of ICI and thus will further enhance the plasticity-inducing potential of DIS for ipsilateral CST like for contralateral CST.

To investigate optimal detection of ipsilateral CSE, five experiments were designed. Additionally, a sixth experiment was designed to enhance ipsilateral CSE. The purpose of the initial experiments was to test for connections using 4 different coil orientations, different muscles being contracted, or different stimulation points being targeted. To test for intracortical properties, influence of different IPIs on ipsilateral CSE was measured. Furthermore, intra-subject retest reliability was tested for ipsilateral CSE. In the sixth experiment, we designed a protocol based on previous findings to enhance ipsilateral CSE. In detail, a DIS protocol (Cash et al., 2016) was paired with MI of finger and wrist extension (Kraus et al., 2016a). To test for associativity of the intervention, DIS was applied while performing a mental calculation task or MI trials were performed with sham TMS (Lisanby et al., 2001). LTP-like effects on ipsilateral CSE were analyzed via the optimal stimulation technique investigated in the first five experiments.

Detection of ipsilateral CSE was significantly increased by contraction of biceps brachii (BB) compared to extensor digitorum communis (EDC) contraction. However, ipsilateral CSE was not modulated by orientation of stimulating coil, point of stimulation, or level of muscle contraction force. Optimal stimulation protocol resulted in a good retest reliability. Furthermore, paired-pulse TMS showed significant changes in the ipsilateral CSE at IPIs of 1.3ms and 220ms. Latency of ipsilateral MEP lagged significantly behind latency of contralateral MEP.

DIS alone significantly increased ipsilateral CSE after the intervention, an observation we did not detect after the combined MI/DIS intervention or MI alone. The observed CSE increase was muscle specific for the extensor muscle and not detectable in the flexor muscle. Furthermore, only DIS resulted in a significant contralateral CSE enhancement, but not MI with DIS or sham TMS, respectively.

Taken together, we optimized the stimulation protocol to elicit reliable ipsilateral MEPs in the EDC, a muscle not studied in detail previously. Furthermore, the ipsilateral CSE can be modulated by paired-pulse TMS like an I-wave generating neuronal network. Finally, a disinhibition protocol facilitated ipsilateral uncrossed CST.

3.3 Motor task dependent modulation of disinhibition stimulation to enhance ipsilateral corticospinal connections for stroke rehabilitation

As patient outcomes are related to initial stroke severity and functionality of contralateral CST from ipsilesional M1 (Stinear et al., 2012), therapeutic models and their investigation are required for patients at the chronic stage (Small et al., 2013; Plow et al., 2016). Furthermore, models taking into account the extent of structural damage and availability of residual motor pathways have been proposed (Bradnam et al., 2013b; Di Pino et al., 2014a; Plow et al., 2016). It was suggested that more affected patients rely on undamaged contralesional motor cortices for recovery (Grefkes and Ward, 2014; Sankarasubramanian et al., 2017; McCambridge et al., 2018). To induce motor recovery, a functional decrease of GABAergic inhibition is favorable (Lazar et al., 2010; Blicher et al., 2015). Intracortical circuits can be targeted via DIS, e.g., modulation of glutamatergic and GABAergic interneurons (Thickbroom et al., 2006; Cash et al., 2016). Another approach to disinhibition is via motor execution. In detail,

unilateral hand movement modulates CSE (Hortobágyi et al., 2003; Zijdewind et al., 2006; Stippich et al., 2007; Perez and Cohen, 2008a; Chiou et al., 2013) and decreases ICI (Goodwill et al., 2012; Chiou et al., 2013). In this study, we analyzed the combination of two disinhibition protocols, each targeting GABAergic interneuron networks, in healthy subjects and severely affected stroke patients. DIS in combination with voluntary motor activity of the non-paretic limb is a potential mechanism to release the contralesional M1 from GABAergic mediated inhibition, thus inducing plasticity not only in contralateral, but also ipsilateral CST.

This study consisted of two experiments. First, we investigated ipsilateral CSE modulation in healthy subjects. The second experiment was performed with severely affected stroke patients. The purpose of both experiments was to enhance ipsilateral CSE. For healthy subjects, we designed a protocol based on previous findings, e.g., combination of DIS (Cash et al., 2016) with contralateral finger and wrist extension. Associativity of the intervention was tested with DIS during flexion or during a mental calculating task (Kanthack et al., 2017). For the patients, we designed a protocol based on results from the healthy subject group to enhance ipsilateral CSE, e.g., DIS in combination with non-paretic finger and wrist extension. To test for placebo effects, the same protocol was applied with the stimulation coil over the occipital cortex (Khedr et al., 2008). LTP-like effects on ipsilateral CSE were analyzed via TMS. Additionally, in stroke patients we measured motor impairment and spasticity of wrist and fingers using Fugl-Meyer (FM) (Fugl-Meyer et al., 1975) and modified Ashworth spasticity (MAS) (Bohannon and Smith, 1987) scales, respectively.

In both healthy subjects and stroke patients, latency of ipsilateral MEP was significantly later than latency of contralateral MEP. DIS combined with wrist/finger extension resulted in a significant increase of ipsilateral CSE. This combination also significantly increased the contralateral MEP. This enhancement was muscle specific and applied only to extensor muscles.

In healthy subjects, DIS combined with wrist/finger flexion resulted in an enhancement at only one time point, e.g., 30 minutes after the intervention, but not immediately.

In stroke patients, we found no change in the FM or MAS. DIS over occipital cortex combined with wrist/finger extension did not result in any changes of CSE.

Taken together, we were able to facilitate uncrossed ipsilateral CST in both healthy subjects and severely affected stroke patients.

4 Discussion

4.1 Corticospinal excitability modulation

Disinhibition of intracortical networks can be an active process to facilitate activity in excitatory neurons that had previously been inhibited, and this plays an important role in resynchronizing and maintaining rhythms in selective neuronal populations (Cash et al., 2010). Although new interventions continue to be developed, the range of stimulation parameters that can be manipulated within a given intervention is considerable and likely to influence the efficacy of intervention in complex ways (Siebner and Rothwell, 2003). Therefore, we investigated the potential of plasticity inducing protocols in detail with the aim to develop an approach towards neurorehabilitation in severely affected patients. In this line of work, disinhibition of

GABAergic-driven ICI by a repetitive paired-pulse TMS paradigm (DIS) (Cash et al., 2016) proved to be a valid foundation for the design of protocols targeting the enhancement of CSE.

Paired pulse TMS enables the targeting of intracortical circuits (Kujirai et al., 1993; Ziemann et al., 1996b). An initial conditioning stimulus evokes multiple descending volleys (I-waves) at a periodicity of about 1.1–1.5ms from transsynaptic activation of principal cells through excitatory interneuronal connections (Di Lazzaro et al., 2012). These I-waves cause a time-dependent modulation of the MEP response to subsequent stimuli and allow targeting of $GABA_B$ergic interneuron networks, resulting in a disinhibition of CSE (Cash et al., 2010, 2016).

Other methods for the inhibition of inhibitory interneurons and therefore disinhibition of CSE include active movement of a limb (Goodwill et al., 2012; Chiou et al., 2013) or imagination of movement (Takemi et al., 2013). Unilateral motor execution has been shown to modulate bilateral CSE (Hortobágyi et al., 2003; Zijdewind et al., 2006; Stippich et al., 2007; Perez and Cohen, 2008a; Chiou et al., 2013) and disinhibit ICI (Goodwill et al., 2012; Chiou et al., 2013). MI modulates GABAergic inhibition (Takemi et al., 2013; Chong and Stinear, 2017).

Here, we analyzed the combination of multiple disinhibition protocols, each targeting GABAergic interneuron networks. Decrease of GABAergic inhibition has been linked to motor learning and recovery (Lazar et al., 2010; Blicher et al., 2015). For contralateral CSE, a combination of DIS with MI proved to be the best combination in inducing strong and persistent enhancement of contralateral CST. However, as contralateral CST is not the optimal reserve for plasticity inducing protocols in severely affected patients (Di Pino et al., 2014b), we next investigated modulation of ipsilateral CST. For ipsilateral CST controlling the wrist extensor muscle EDC, we showed for the first time a reliable detection of ipsilateral MEPs. Furthermore, to our knowledge no previous study has investigated the intracortical properties with regard to facilitation and disinhibition. We were able to show that ipsilateral CST, like contralateral CST, is modulated by intracortical circuits. This finding indicates DIS as a valid protocol for targeting disinhibition of the ipsilateral CST. In line with these results, we were able to facilitate ipsilateral CSE via DIS. Next, we combined DIS with active motor execution, e.g., active extension of contralateral wrist and fingers in healthy subjects or active extension of non-paretic wrist and fingers in stroke patients. This combination resulted in a significant and persistent increase of ipsilateral CSE in both healthy subjects and severely affected stroke patients.

4.2 Contralateral corticospinal excitability

Contralateral CSE was significantly increased by the combination of two disinhibition protocols, each targeting interneuron networks. DIS modulates CSE excitability by targeting glutamatergic transmission and $GABA_B$ receptors (Cash et al., 2016). MI improves excitatory synapse efficiency via postsynaptic $GABA_A$ and presynaptic $GABA_B$ receptor inhibition (Abbruzzese et al., 1999; Stinear and Byblow, 2004a; Takemi et al., 2013; Chong and Stinear, 2017). Furthermore, MI leads to bilateral ERD (Vukelić et al., 2014) and activates muscle specific excitatory neurons (Takemi et al., 2013).

Inhibition of $GABA_B$ receptors by paired-pulse TMS was initially shown to occur at rest (Cash et al., 2010, 2011). However, this observation conflicts with recent data. In detail, paired-pulse TMS mediated LCD was not detected at rest, but only during MI

(Chong and Stinear, 2017) or voluntary muscle contraction (Caux-Dedeystère et al., 2015). These observations are in line with our findings, as only combination of DIS and MI resulted in significant and persistent increases of contralateral CSE. Improved excitatory synaptic transmission during MI may thereby have acted as a gating mechanism to enhance the plasticity inducing effects of DIS.

The observed effects point towards synergistic mechanisms. Specifically, as both disinhibition protocols target $GABA_B$ergic transmission, stronger simultaneous disinhibition may have been the important factor. This is supported by the known task-related modulations of physiological characteristics in cortical interneuronal populations (Murthy and Fetz, 1996) and the link between GABAergic disinhibition and CSE modulation (Lazar et al., 2010; Blicher et al., 2015).

Therefore, task-related modulation and timing of TMS to optimal brain state is of high importance; an observation further supported by our finding that only associative pairing of MI and DIS resulted in LTP-like plasticity, i.e., DIS during MI but not after. This is in line with previous results from brain-state dependent stimulation protocols (Gharabaghi, 2015; Kraus et al., 2016b, 2018; Royter and Gharabaghi, 2016), e.g., stimulation during MI results in an increase of CSE that lasts beyond stimulation while a stimulation independent of state results in a decrease (Kraus et al., 2016a).

Neuromuscular ES did not amplify the effects of investigated disinhibition protocols on contralateral CSE after the intervention in our study. This observation is in contrast to previous studies showing an enhanced ERD (Reynolds et al., 2015) and CSE (Kaneko et al., 2014) in combination with MI. However, these effects could only be shown during the intervention and did not result in persistent changes after intervention. Furthermore, ES does not stimulate only motor nerve fibers but also afferent sensory nerve fibers. Alteration of afferent input has been shown to lead to organizational changes in the cortex in rodents (Sanes et al., 1992), and prolonged stimulation of peripheral nerves can induce changes in motor networks in the human cortex (Ridding et al., 2000). The lack of plasticity induction may be due to different reasons, e.g., insufficient intervention dose or inadequate temporal pairing of the feedback stimuli (ES and TMS). Furthermore, as ES cannot be modulated contingently by the level of MI-related ERD and follows an all-or-nothing principle of muscular activation, stimulation during a non-favorable brain state might have occurred. Contingent feedback based on MI-related ERD can be achieved via robotic orthoses within brain-machine interfaces (BMIs) (Bauer et al., 2015; Brauchle et al., 2015; Vukelić and Gharabaghi, 2015) and might thereby represent an alternative approach towards sensory feedback. This suggestion is further supported by findings indicating 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 (Naros et al., 2016).

The range of stimulation parameters that can be manipulated is considerable and likely to influence the efficacy of the intervention (Siebner and Rothwell, 2003), and ES could enhance motor recovery on the grounds that it simulates a meaningful task and increases functional relevance (Rushton, 2003). Therefore, afferent feedback provided by ES coupled with task-related motor execution instead of MI could facilitate CSE and requires further investigation. However, active motor execution of paretic muscles is

not an option for severely affected stroke patients and other feedback modalities like robotic orthoses within BMI might be the preferred direction for future investigations.

4.3 Ipsilateral corticospinal excitability

Although most corticospinal fibers decussate to form the contralateral CST, ipsilateral pathways forming the ipsilateral CST exist (Gerloff et al., 1998; Brus-Ramer et al., 2009; Bradnam et al., 2013a; Wahl et al., 2017). TMS of M1 can probe both contralateral and ipsilateral CST via MEPs (Ziemann et al., 1999; Chen et al., 2008). The optimal protocol for eliciting ipsilateral MEPs in the EDC was to choose the contralateral hotspot (45° coil orientation) and stimulate at a high stimulator output intensity with BB being contracted. This contrasts with previous findings showing that ipsilateral MEPs had a different preferred current direction and stimulation point for activation when compared with contralateral MEPs (Ziemann et al., 1999; Alagona et al., 2001). However, more recent data also showed no difference in spatial location of the ipsilateral and contralateral center of gravity (MacKinnon et al., 2004). The observed discrepancies might be due to different muscles being investigated, as proximal and distal arm muscle representations differ in morphological and functional differences, e.g., transcallosal projections (Pandya and Vignolo, 1971; Jenny, 1979; Gould et al., 1986). We observed an increased ipsilateral MEP probability for the EDC muscle, when BB and not EDC was pre-activated. A possible explanation for this finding is the dependence of activity in ipsilateral pyramidal neurons on type of upper-limb movement (Tanji et al., 1988; Cisek et al., 2003). These findings further underline the importance of characterizing the muscle of interest in detail before investigating potential changes in ipsilateral CSE due to a plasticity enhancing intervention.

Furthermore, the reliability of the assessment tool is an important criterion to ensure that the changes observed are in relation to physiological changes and not due to chance. Whereas extensive data on contralateral MEP reliability are available (Bastani and Jaberzadeh, 2012; Goldsworthy et al., 2016; Kraus and Gharabaghi, 2016), to our knowledge, no study has investigated ipsilateral MEP reliability. We detected good inter-session reliability of ipsilateral MEP measurements. Additionally, the intraclass correlation coefficient was similar to the one of contralateral MEPs, which are accepted as a reliable outcome measure to characterize properties of the contralateral CST (Di Lazzaro et al., 2004, 2012; Ziemann et al., 2015).

Latency of ipsilateral MEP differed significantly from latency of contralateral MEP. Although the differences may be slight underestimates due to muscle activation (Alagona et al., 2001), we report a similar lag of ipsilateral latency as previous research (Ziemann et al., 1999; Bradnam et al., 2010; Tazoe and Perez, 2014; McCambridge et al., 2016). The delayed latency points towards involvement of an oligosynaptic pathway or a longer conduction distance from the excited cortical area to the origin of its descending pathway. Different descending pathways originating in the medial brainstem are plausible, e.g., reticulospinal (Riddle et al., 2009; Baker and Perez, 2017) or rubrospinal pathway (Ishida et al., 2016). Another possibility are the midline-crossing fibers of contralateral CST (Starkey et al., 2012; Wahl et al., 2017). Whereas, a modulation by the opposite hemisphere is unlikely as transcallosal activation requires approximately 10-20ms (Cracco et al., 1989; Meyer et al., 1998) and is therefore too long to account for the latency difference.

Paired pulse TMS enables the assessment of intracortical excitatory and inhibitory circuits (Kujirai et al., 1993; Ziemann et al., 1996b). Whereas inhibitory networks of ipsilateral CST have been analyzed before (McCambridge et al., 2016), to

our knowledge, we are the first to show that SICF and LCD can also be measured for ipsilateral CST. As facilitation of the contralateral CST is dependent on I-wave generating neurons (Ziemann et al., 1998; Ilić et al., 2002; Thickbroom, 2011), we can assume that the ipsilateral pathway is also modulated by I-wave generating neurons. In comparison to previous studies (McCambridge et al., 2016), we were not able to detect an inhibitory effect. However, studies of contralateral CST showed a relationship between the degree of ICI and priming stimulus intensity as an inverted U-shaped curve (Chen et al., 1998; Ilić et al., 2002). The high stimulator output intensity used as the priming stimulus in our protocol may have led to reduction of inhibition and subsequent facilitation. Additionally, the time course of inhibitory and facilitatory effects varies in a task-dependent manner (Caux-Dedeystère et al., 2014) and could therefore be modified by BB preactivation. It remains unclear if facilitation is mediated in the exact manner as for contralateral CST, e.g., activation of glutamatergic interneurons for SICF (Ziemann et al., 1998; Ilić et al., 2002) and $GABA_B$ mediated disinhibition for LCD (Cash et al., 2011). Nevertheless, the comparable findings for contralateral and ipsilateral CST and therefore analogous intracortical properties proved to be a solid premise for modulating ipsilateral CSE via DIS.

For ipsilateral CSE, DIS stimulation alone or in combination with voluntary motor execution of contralateral EDC, but not in combination with MI, resulted in a significant plasticity induction.

This contrasts with the observed plasticity inducing effect in the contralateral CSE, e.g., only a combination of MI and DIS resulted in a persistent increase of contralateral CSE. Whereas bilateral ERD during MI (Vukelić et al., 2014) and modulation of GABAergic intracortical neuronal populations (Takemi et al., 2013; Chong and Stinear, 2017) appeared to favor plasticity induction of contralateral CSE, other mechanisms might have inhibited the efficiency of intracortical network disinhibition for ipsilateral CSE. Besides bilateral ERD, MI also results in IHI (Liang et al., 2008, 2014; Perez and Cohen, 2008b). IHI involves activity across the corpus callosum through dense projections (Ferbert et al., 1992; Meyer et al., 1995) and is mediated by a decrease of the last I-wave (Di Lazzaro et al., 1999). Additionally, an involvement of transcallosal glutamatergic pathways linking with pyramidal tract neurons through $GABA_B$-mediated inhibitory neurons has been proposed (Ferbert et al., 1992; Reis et al., 2008; Perez and Cohen, 2009). ICI and IHI affect each other in a negative feedback loop (Sanger et al., 2001; Carson, 2005), e.g., an increase in IHI results in a decrease of intracortical inhibition and vice versa (Daskalakis et al., 2002; Lee et al., 2007). MI-mediated IHI might have suppressed excitability of cortical output neurons more strongly than it disinhibited intracortical neuronal population. Decreased excitability and increased $GABA_B$-mediated inhibition, in turn, might have silenced the plasticity inducing efficiency of DIS for not only ipsilateral, but also contralateral CSE.

To bypass the negative effect of IHI on ipsilateral CSE while still maintaining the positive effect of ERD observed on contralateral CSE, we combined DIS with a voluntary motor execution of contralateral wrist extensor. For instance, activation of contralateral right EDC leads to an ERD of the left M1, which, in turn, is favorable for ipsilateral CSE of left EDC, and thereby for plasticity inducing effects of DIS. Specifically, a decrease of IHI from the opposite cortex (Liang et al., 2011) and a disinhibition of intracortical circuits (Muellbacher et al., 2000; Perez and Cohen, 2008a) may be induced by activating the contralateral side, resulting in an increased facilitation of ipsilateral CSE. This hypothesis is supported by our findings, as contralateral muscle activation resulted in an enhanced CSE of the ipsilateral homologous muscle.

4.4 Future perspectives

The functional relevance of our physiological findings should be tested in more detail with regard to behavioral outcome parameters along the lines of previous protocols. The latter indicate a correlation between the magnitude of induced plasticity and voluntary motor output in both injured and healthy subjects (Taylor and Martin, 2009; Bunday and Perez, 2012). Additionally, future studies need to investigate the sustainability of plasticity induction for longer follow-up periods, particularly since previous work indicate that CSE increases of DIS last for no longer than 60 minutes (Cash et al., 2016). We found significant changes in the contralateral or ipsilateral CSE for up to 30 or 60 minutes, respectively. However, we did not assess changes in CSE at a later time point. Changes of corticospinal transmission are functionally relevant in both healthy (Taylor and Martin, 2009) and injured conditions (Bunday and Perez, 2012). Conversely, we did not observe any differences in motor behavior in patients. This contrasts with findings of a positive correlation between PAS-induced CSE increases and enhancements in voluntary motor output in both healthy and injured subjects, indicating an association between motor output and magnitude of induced plasticity (Bunday and Perez, 2012). A possible explanation for the missing effect on voluntary motor output might be the short duration of intervention (1.5min). A therapeutic intervention over a longer duration, even over days, may result in similar positive changes of not only ipsilateral CSE, but also motor output in the patient. This is supported by the observation that clinical effects are often short-lived and multiple intervention sessions seem to extend clinical benefit (Bäumer et al., 2003; Edwards et al., 2008). The required duration as well as the frequency of application need to be further addressed in future studies. However, when designing a protocol, one has to keep in mind that increased stimulus dosage appears to also increase the potential risk of adverse events, including seizure and headache (Rossi et al., 2009).

We showed that individually tailoring IPI and IDI resulted in significant online increases of CSE but not in changes in offline increases. Furthermore, studies investigating the optimization of intervals to individual optimum showed significant changes also after the intervention (Sewerin et al., 2011; Cash et al., 2016). However, when optimal interval for paired-pulse TMS was not detectable during the session in patients, we chose intervals based on our previous results. This approach could have limited ipsilateral CSE facilitation. Future studies should therefore first include a screening session, followed by the interventional session to ensure optimization of intervals for each patient.

Another limitation of this work is that we studied the optimal protocol for detection of ipsilateral CST only in healthy subjects. Furthermore, reliability tests of CSE were not performed for stroke patients. Therefore, it remains unclear if the same properties of the ipsilateral CST and the high inter-session reliability can be observed in affected patients, e.g., stroke patients. Nevertheless, we do not expect any changes in orientation of interneuronal networks. Moreover, as ipsilateral CSE was modulated in stroke patients, we can assume that the same intracortical properties have been targeted by DIS.

DIS in combination with wrist/finger extension may have the potential to adjust the brain into a more receptive (e.g., plastic) state. This receptive state could then be used as a time point for physiotherapy and further improve clinical outcome (Talelli and Rothwell, 2006; Edwards et al., 2008).

4.5 Conclusion

The different approaches needed based on consideration of structural damage and availability of residual motor pathways (Bradnam et al., 2013b; Di Pino et al., 2014a; Plow et al., 2016) further highlight the importance of CST being investigated.

For contralateral CST, combining endogenous with exogenous disinhibition of intracortical circuits 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, and ipsilesional CST is still functional enough to be targeted.

If ipsilesional CST is too severely damaged, contralesional motor cortices have to be targeted. Ipsilateral MEPs can be detected in the EDC muscle with good reliability and be modulated by intracortical circuits, thereby providing a premise for disinhibition protocols. DIS in combination with voluntary motor activity of the non-paretic limb is a potential mechanism to release the contralesional M1 from GABAergic mediated inhibition and thus induce lasting plasticity of ipsilateral CST. These findings may therefore provide a therapeutic backdoor using interventions to modulate the ipsilateral pathway and activating normally inhibited routes when contralateral connections are no longer a viable target for plasticity enhancing interventions, e.g., in severely affected stroke patients.

The magnitude of plasticity induction needs to be increased to maximize efficacy, while intervention length should be reduced to facilitate transfer to clinical application. DIS provides a prospective building block for potential therapeutic interventions and requires further investigation in a larger stroke patient cohort in order to fully verify lasting plasticity induction and changes in motor behaviour outcome.

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6 List of appended manuscripts and contributions

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

Ziegler L; Schulte R; Gharabaghi A

Submitted Brain Stimulation

Contributions:

L.Z.: study design, data acquisition, data analysis, writing the manuscript

A.G.: study design, data analysis, writing the manuscript

R.S.: data acquisition (Experiment 3), data analysis (Experiment 3)

6.2 Detection and enhancement of ipsilateral connections in the human brain

Ziegler L; Trunk B; Meinen A; Gharabaghi A

Intended submission to Journal of Neuroscience

Contributions:

L.Z.: study design, data acquisition, data analysis, writing the manuscript

B.T.: data acquisition (Experiment 5)

A.M.: data acquisition (Experiment 5)

A.G.: study design, data analysis, writing the manuscript

6.3 Motor task dependent modulation of disinhibition stimulation to enhance ipsilateral corticospinal connections for stroke rehabilitation

Ziegler L; Trunk B; Kammermeier P; Gharabaghi A

Intended submission to Journal of Neuroscience

Contributions:

L.Z.: study design, data acquisition, data analysis, writing the manuscript

B.T.: data acquisition (healthy subjects)

P.K.: data acquisition (healthy subjects)

A.G.: study design, data analysis, writing the manuscript

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8 Appendix

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

Title

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.

Objective

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 of finger extension with a disinhibition protocol (DIS) targeting intracortical circuits in the respective cortical motor representation by paired-pulse repetitive transcranial magnetic stimulation.

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 individualized 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 applying concurrent neuromuscular electrical stimulation the plasticity induction was cancelled.

Conclusions

A novel associative stimulation protocol augments plasticity induction in the human motor cortex within a remarkably short period of time and in the absence of active movements. Combining 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.

Keywords

Motor Cortex; Motor Imagery; Transcranial Magnetic Stimulation; Electrical Stimulation; Motor Evoked Potentials; Disinhibition

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 [10]). A novel paired-pulse rTMS protocol, 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 has been 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 do usually 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. 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 we also investigated the modulatory influence of individually adjusted IPI and IDI intervals, of an expanded intervention period (480 instead of 48 stimuli) and of concurrent neuromuscular electrical stimulation (ES) to the targeted muscle.

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 avoid 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] and no 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 Figure 1.

The number of subjects in each experiment differed due to dropouts 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.

Experiment 1 consisted of four conditions (MI/DIS, MI_{OFF}/DIS, DIS, and MI): A DIS protocol [11] (see below for details) was paired with MI of finger and wrist extension [39] (see below for details). On the basis of previous findings [11] DIS was applied with the same interpulse interval (IPI; 1.3 ms) and interdoublet interval (IDI; 220 ms) in all subjects. Four stimulation doublets (i.e., eight stimuli) were applied at the time of imagined movement (during the MI_{ON} phase) in each trial. In all, 48 stimuli were applied during six MI trials (MI/DIS condition). To test for associativity of the intervention, the same DIS protocol was applied during the MI_{OFF} phase, i.e., *between* the time of the imagined movements (MI_{OFF}/DIS condition), or during REST, i.e., *without* a motor imagery task (DIS condition). Moreover, the six MI trials were also performed *without* any DIS (MI condition).

In the follow-up Experiments 2-4, the IPI and IDI intervals were individually adjusted for each subject and session to further enhance the stimulation effect on CSE [13].

The follow-up Experiment 2 consisted of four conditions: MI/DIS/ES, MI_{OFF}/DIS/ES, DIS/ES, and MI/ES. These were similar to those in Experiment 1, but with simultaneous ES application. ES lasted 3 s and the DIS was applied halfway through. ES was included in the experimental design since it had been shown to increase the effects of single TMS pulses on CSE [40,41]. It was also known to enhance MI-related ERD [27] and to increase CSE to a greater extent than MI itself [28].

The follow-up Experiment 3 consisted of four conditions: MI/DIS, MI/DIS_{10}, DIS_{10}, and MI_{10}: These were similar to the conditions in 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]. MI_{OFF}/DIS was not further considered due to the negative findings in Experiments 1 and 2.

The follow-up Experiment 4 consisted of five conditions: MI/DIS_{10}, MI/DIS_{D10}, and MI/ES_{10}, MI/ES+DIS_{10}, MI/DIS+ES_{10}. On the basis of recent findings of state-dependent interventions [39,43], stimulation bursts were applied only when a predefined ERD threshold (see below) was achieved during MI to improve the associativity with MI-related ERD. This resulted in varying inter-burst intervals and in a mean of 98.6 ± 16.2 MI trials per condition to obtain 60 trials in which the predefined ERD threshold was exceeded to trigger the stimulation. The conditions were as in Experiment 2 (MI/DIS/ES condition), but with a 10x expanded number of cortical stimuli (480 pulses in all) to match the number applied during Experiment 3. Experiment 4 differed from Experiment 2 in that DIS and ES were applied subsequently instead of simultaneously, i.e.,, DIS+ES or ES+DIS. This modification of the study design was based on the negative findings of the simultaneous DIS/ES application in Experiment 2 and on previous research on subsequent PAS [41,44]. In one additional condition (MI/DIS_{D10}), the timing of DIS was *delayed* to match the timing of DIS during the ES+DIS condition and to avoid a timing-depended bias.

The number of delivered TMS pulses during pre/post evaluation differed between experiments: In all Experiments, 40 stimuli were applied at 40 % MSO before the intervention. For the detection of optimal IPI and IDI in Experiments 2-4, 10 paired-pulse stimuli at 110 % RMT were applied before the intervention at intervals of 1.1, 1.2, 1.3, 1.4, 1.5, 150, 200, 220, 230, and 250 ms, respectively. Additionally, 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, before and after the intervention (twice after the intervention for Experiments 1 and 2; 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).

The material and methods of data acquisition applied here were identical to those of previous studies [39,45] and have been cited accordingly:

Data Acquisition

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 online analysis to MATLAB, where they were stored for offline 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).

Pre/post TMS protocol

A biphasic TMS pulse (MagPro-R30 + MagOption, MagVenture GmbH, Germany) was applied 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 neuro-navigation (TMS Navigator, Localite GmbH, Germany) supported the localization of the TMS target position. Once a template MRI (MNI ICBM152 non-linear symmetric T1 Average Brain) had been registered to the head of each participant, the neuro-navigation system tracked the relative positions of the TMS coil and the participant's head during the experiment whilst keeping the stimulation location constant [47]. Subjects were seated comfortably in an armchair with the 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]. We used 40% of maximum stimulator output as the initial intensity applied to the anatomically defined 'hand knob' of M1. Whenever the initial stimulator output did not suffice to elicit MEPs, we increased the output in steps of 5%. We ensured that the orientation of the coil remained perpendicular to the central sulcus, and the coil site that consistently elicited the largest MEPs was defined 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].

Disinhibition stimulation (DIS)

Cortical stimulation was performed on the basis of a paired-pulse rTMS protocol which – for the sake of simplicity and in accordance with previous literature that took the same approach [11] – will be referred to as disinhibition stimulation in this manuscript. 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]. Specifically, IPIs of 1.1, 1.2, 1.3, 1.4, and 1.5 ms, and IDIs of 150, 200, 220, 230, and 250 ms were investigated for every subject at the beginning of each experiment [11].

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, Hasomed GmbH, 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.

Motor imagery (MI)

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. In Experiment 4, at least four runs, each consisting of fifteen trials, were performed until 60 trials with sufficient ERD and triggered stimulation were obtained (Figure 1A).

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 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 increases when MI and ES occur simultaneously, but not when the latter is triggered by the former [27].

ERD detection

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. 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 (DIS/ES; ES/DIS; DIS) was triggered only if an event-related desynchronization (ERD) was observed in the β-band (16–22 Hz) during the MI phase [58]. ERD detection was confined to electrodes FC4, C4 and CP4 over the right sensorimotor area [59]. 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) to detect decreases in sensorimotor rhythm (SMR) 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 event-related synchronization (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 in each subject throughout the intervention.

MEP evaluation during the intervention

MEP amplitudes elicited by doublet stimulation were measured to assess changes in CSE during the intervention induced by the condition. We inspected the EMG data

during offline analysis, discarding any trials containing muscle pre-activation (rectified pre-stimulus EMG activity above 20 µV). Due to the stimulation artifact during ES, DIS conditions which contained simultaneous ES could not be analyzed with regard to MEP amplitudes during the intervention. MEP amplitudes elicited by doublet stimulation during the intervention were averaged on a run-to-run basis for the following statistical analysis (see paragraph *Statistical Analysis*).

CSE analysis

To study CSE before and after the interventions, TMS pulses were triggered every 5 s (±1.25 s predefined jitter).
For Experiments 1, 2, 3, and 4, we tested the MEP stimulus-response curve using a range between 110-150 % RMT in 10 % steps to determine CSE at baseline (prior to intervention) and after the intervention. 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. The lasting effects were determined differently. 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 determine time-dependent changes of plasticity. In Experiment 3, the measurement was performed at 15, 30, 45, and 60 min post-intervention, and at 15, 30, 40, 50, and 60 min post-intervention in Experiment 4.
Furthermore, we acquired a cortical map representation at 110% RMT for a virtual grid 30 minutes after the intervention. In Experiment 1, a 7-by-7 grid (5 x 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 x 5 mm per cell) in Experiments 2-4. The cortical map was assessed by a random order stimulation of all points to avoid any carry-over effect.
We inspected the EMG data during offline analysis, 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. The artifact-free MEP amplitudes were then measured peak-to-peak and 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 offline using custom written scripts in MATLAB (R2017a, The MathWorks, Inc., United States).

Statistical analysis

Statistical analysis was performed using statistical functions in MATLAB (R2017a, The MathWorks, Inc., United States). Data sets 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. 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

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 *MEP*.

Online effects

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 effects

To investigate intervention effects on the dependent variable *MEP* measured with the stimulus-response curve, we performed an ANOVA for each applied 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.

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 brain-state 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 de-meaned EMG signal of the MI and the MI_{OFF} period was considered. 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).

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 (Figure 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* (Figure 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 (Figure 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, 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 up to 241.8 ± 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 ± 11.1 % compared to MI (130.4 ± 11.5 %; p=0.034; Tukey's test), DIS (127.9 ± 8.3 %; p=0.019; Tukey's test), and MI_{OFF}/DIS (138.6 ± 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). The maximum SICF occurred at an IPI of 1.1 ms (n = 3), 1.2 ms (n = 6), 1.3 ms (n = 9), 1.4 ms (n = 6), or 1.5 ms (n = 5). The maximum LCD was measured at an IDI of 200 ms (n = 7), 220 ms (n = 11), 230 ms (n = 5), or 250 ms (n = 6). 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).

Experiment 2:

Concurrent neuromuscular electrical stimulation (ES) to the finger extension muscle targeted by MI/DIS cancelled out the consistent CSE increases across 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 (Figure 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:

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

Specifically, ANOVA revealed a significant online effect of *condition* on the mean MEPs (Figure 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 in the course of 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 (Figure 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 immediately after the intervention (Figure 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 compared 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).

Similar to 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 with variable inter-burst periods 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 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 for 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 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 (Figure 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/DIS_{D10} resulted in a significant increase to 130.2 ± 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 significantly differ 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 [41,43,58]. 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 observed plasticity induction 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 an association 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 [41,43,45]. 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

Of the associative protocols investigated here, MI/DIS amplified CSE to ~150-200% of baseline following a ~90 s intervention. Similar effect sizes have already been reported when the same DIS protocol was applied during rest [11]. However, 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. 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 (Figure 4B). The specific stimulation intensities resulting in DIS-induced plasticity at rest may depend on the investigated target muscles (which differ in this and previous studies) or the different inter-burst intervals (13 s in this study vs. 8 s in the study by [11]). Present findings suggest, however, 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].

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]. Our current findings suggest that early and later I-waves may be differently addressed by DIS when comparing unified (Figure 4) to individualized IPI (Figure 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 via the recruitment of early I-waves [79]. When paired with MI, however, the amplified CSE was more consistent across different stimulation intensities (via the recruitment of early and late I-waves). The MEP changes measured during the intervention indicate a CSE baseline shift during MI in comparison to MI_{OFF} or REST (Figure 2). MI appears to modulate the susceptibility of the stimulated intracortical circuits to an excitatory drive similar to a gating mechanism [83].

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 in general not result in associative plasticity after 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) [53,56,84]. 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 revealed, moreover, that individually adjusting IPI and IDI for each subject 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 after the intervention. This may be due 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 close to 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 quite 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, when preselected adequately, unified parameters will result in strong CSE increases in the majority of subjects, thereby limiting the additional benefit of individualization. However, expanding the intervention period improved the sustainability of the plasticity throughout the follow-up period, but did not increase the maximum CSE in comparison to the short intervention period (Figure 7).

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 (Figure 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.

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 for no longer than 60 minutes [11]. Furthermore, the functional relevance of our physiological findings should be tested with regard to behavioral outcome parameters along the lines 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, an extension of the investigated range beyond 1.5 ms should be considered in future studies.

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 might 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 presented findings 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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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. N.S. stands for non-significant.

Experiment	Main Intervention	Stimulus-response curve (stimulation at % RMT)		CSE at 110% RMT (minutes post intervention)
		15 minutes	60 minutes	
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/DIS (15, 30); **MI/DIS_{10} (15, 30, 45, 60)**

		MI_{10} (110)		
4	triggered $MI/DIS/ES_{10}$	n.s.	-	MI/DIS_{D10} (30)

Figure 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 MI_{OFF} phase (MI_{OFF}/DIS) or during REST (DIS). Furthermore, the six MI trials were performed *without* any DIS (MI).

B: Experiment 2 consisted of four conditions: MI/DIS/ES, MI_{OFF}/DIS/ES, DIS/ES, and MI/ES. Each condition had one run containing six trials. ES lasted 3 s and the DIS was applied halfway through.

C: Experiment 3 consisted of four conditions: MI/DIS, MI/DIS_{10}, DIS_{10}, and MI_{10}. MI/DIS had one run containing six trials. The other conditions had four runs á 15 trials.

D: Experiment 4 consisted of five conditions: $MI/ES+DIS_{10}$, $MI/DIS+ES_{10}$, MI/DIS_{10}, $MI/DISD_{10}$ and MI/ES_{10}. Stimulation bursts were applied only when a predefined ERD threshold was achieved until 60 successful trials were obtained. In one additional condition (MI/DIS_{D10}), the timing of DIS was *delayed* to match the timing of DIS during the ES+DIS condition.

Figure 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/DIS_{10} resulted in a significant increase of the mean MEP amplitude ± SEM compared to DIS_{10}. Moreover, both MI/DIS_{10} and DIS_{10} 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).

Figure 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 ($p=0.086$).

Figure 4: *Input-output changes of MEP amplitude normalized to baseline for Experiment 1.*

A Of all the conditions, 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).

Figure 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).

Figure 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).

Figure 7: *Time-course of MEP amplitude changes post intervention for Experiment 3.*

MI/DIS and MI/DIS_{10} showed a significant mean MEP amplitude ± SEM increase immediately after the intervention, but only MI/DIS_{10} significantly increased MEP amplitudes up to the follow-up of 60 min (* indicates $p < 0.05$, Tukey's test).

Figure 8: *Time-course of MEP amplitude changes post intervention for Experiment 4.*

MI/DIS_{D10} led to a significant increase compared to the baseline 30 min after the intervention (* indicates $p < 0.05$, Tukey's test).

Figure 1:

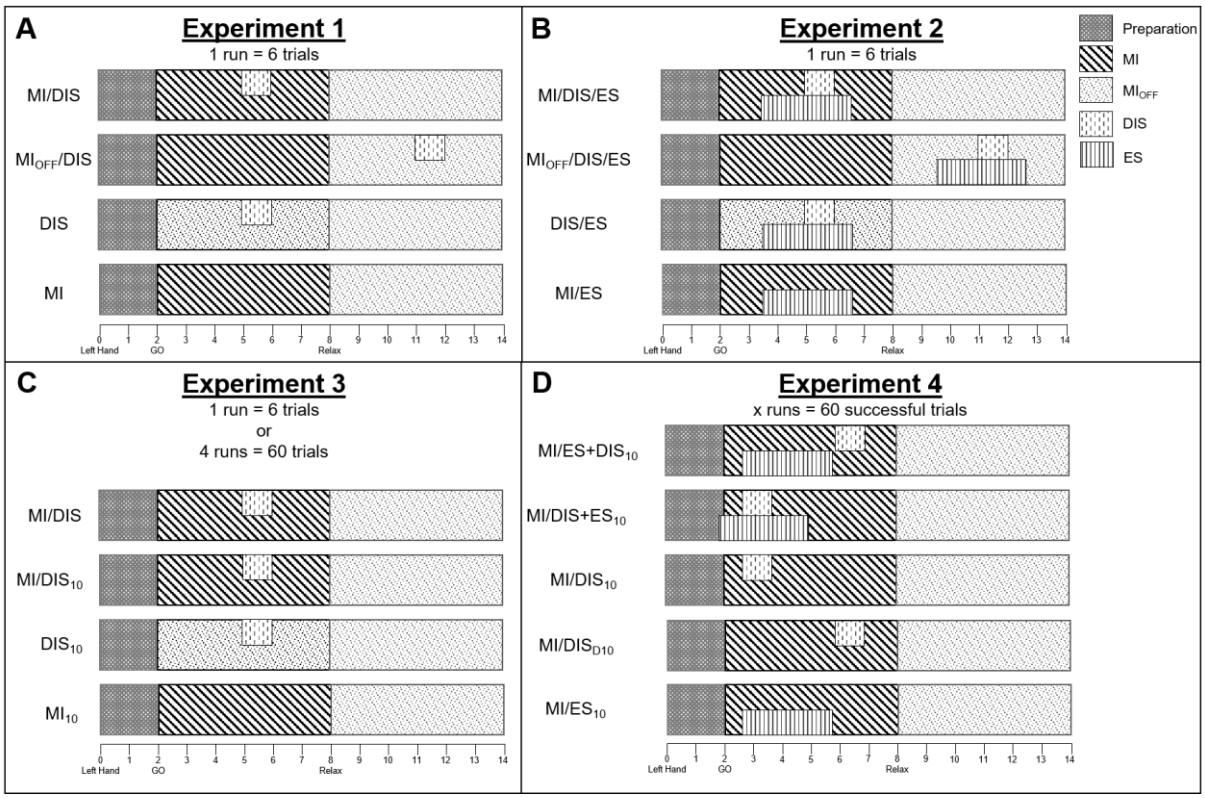

A
Experiment 1
1 run = 6 trials
MI/DIS
MI$_{OFF}$/DIS
DIS
MI
B
Experiment 2
1 run = 6 trials
MI/DIS/ES
MI$_{OFF}$/DIS/ES
DIS/ES
MI/ES
Preparation
MI
MI$_{OFF}$
DIS
ES
C
Experiment 3
1 run = 6 trials
or
4 runs = 60 trials
MI/DIS
MI/DIS$_{10}$
DIS$_{10}$
MI$_{10}$
D
Experiment 4
x runs = 60 successful trials
MI/ES+DIS$_{10}$
MI/DIS+ES$_{10}$
MI/DIS$_{10}$
MI/DIS$_{D10}$
MI/ES$_{10}$
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Left Hand
GO
Relax

Figure 2:

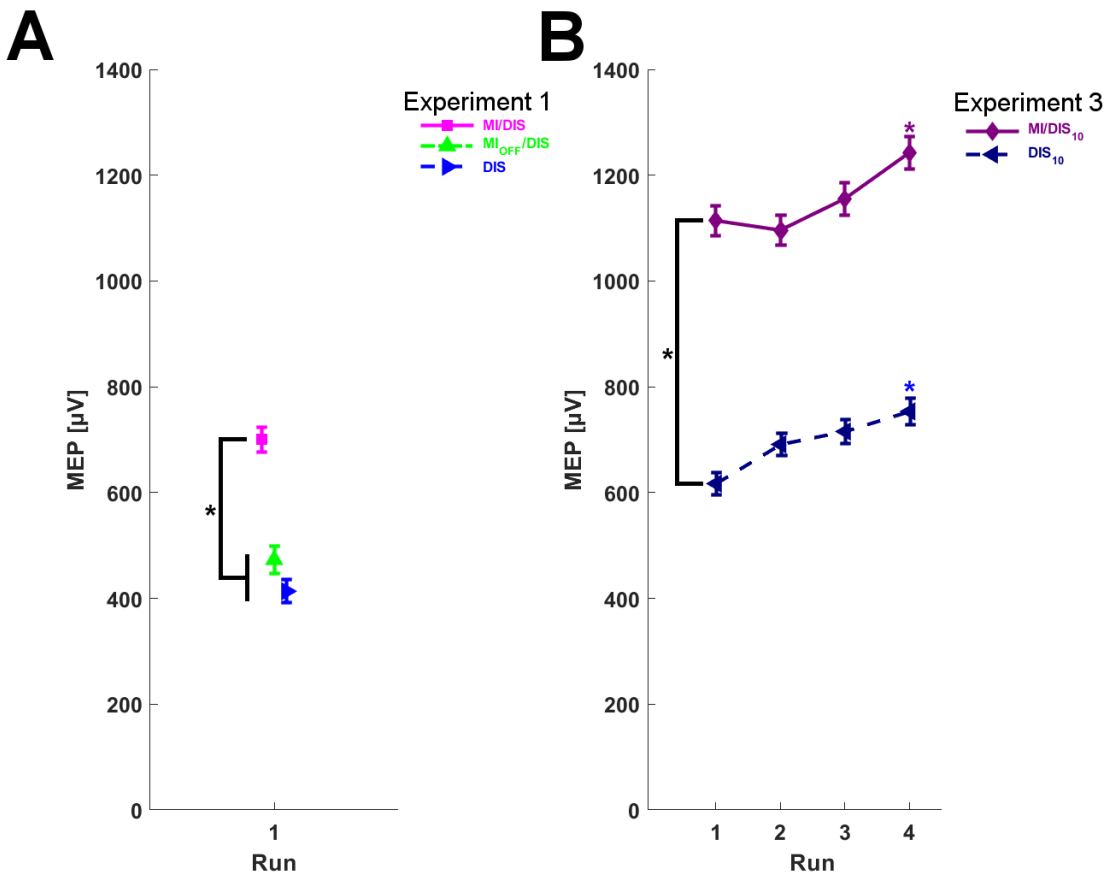

A
B
Experiment 1
MI/DIS
MI_OFF/DIS
DIS
Experiment 3
MI/DIS_10
DIS_10
MEP [µV]
Run
1400
1200
1000
800
600
400
200
0
1
2
3
4
*

Figure 3:

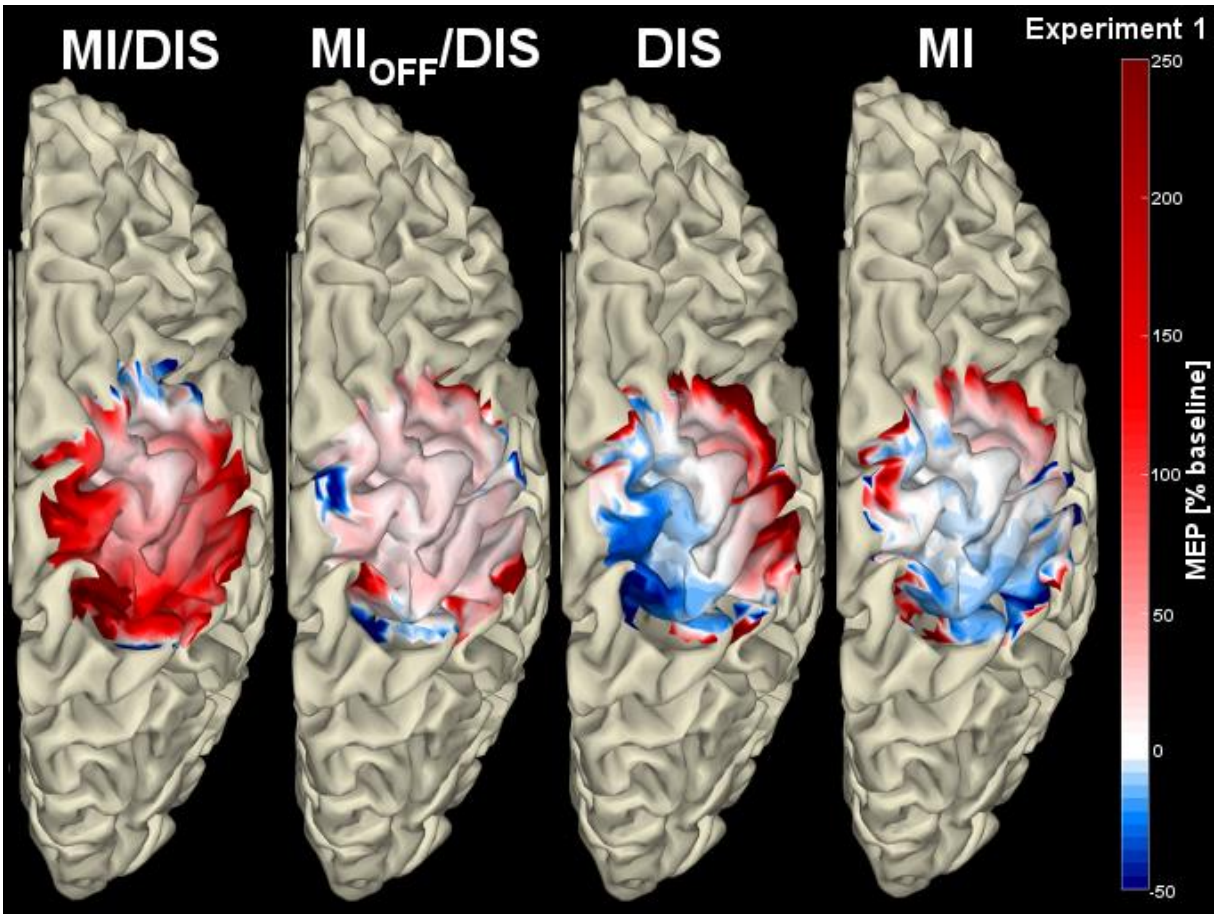

MI/DIS
MI$_{OFF}$/DIS
DIS
MI
Experiment 1
250
200
150
100
50
0
-50
MEP [% baseline]

Figure 4:

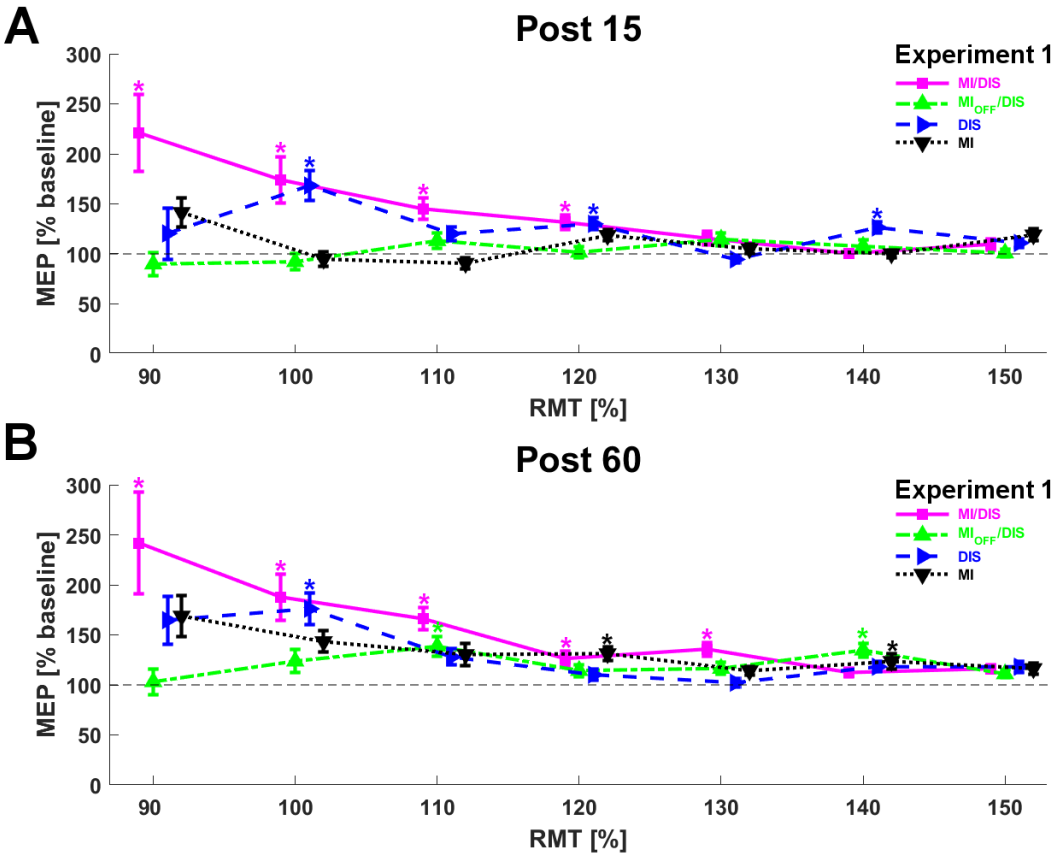

A
Post 15
Experiment 1
MI/DIS
MI_OFF/DIS
DIS
MI
MEP [% baseline]
RMT [%]
B
Post 60
Experiment 1
MI/DIS
MI_OFF/DIS
DIS
MI
MEP [% baseline]
RMT [%]

Figure 5:

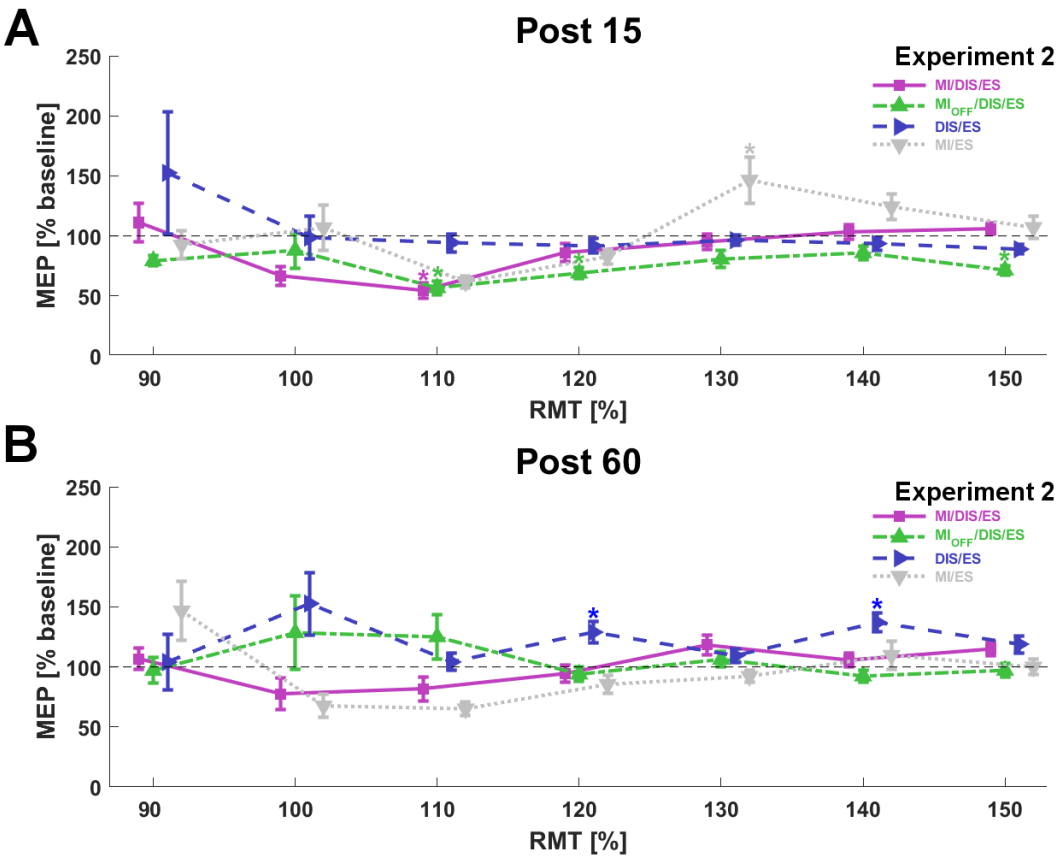

A
Post 15
Experiment 2
MI/DIS/ES
MI$_{OFF}$/DIS/ES
DIS/ES
MI/ES
MEP [% baseline]
RMT [%]
B
Post 60
Experiment 2
MI/DIS/ES
MI$_{OFF}$/DIS/ES
DIS/ES
MI/ES
MEP [% baseline]
RMT [%]

Figure 6:

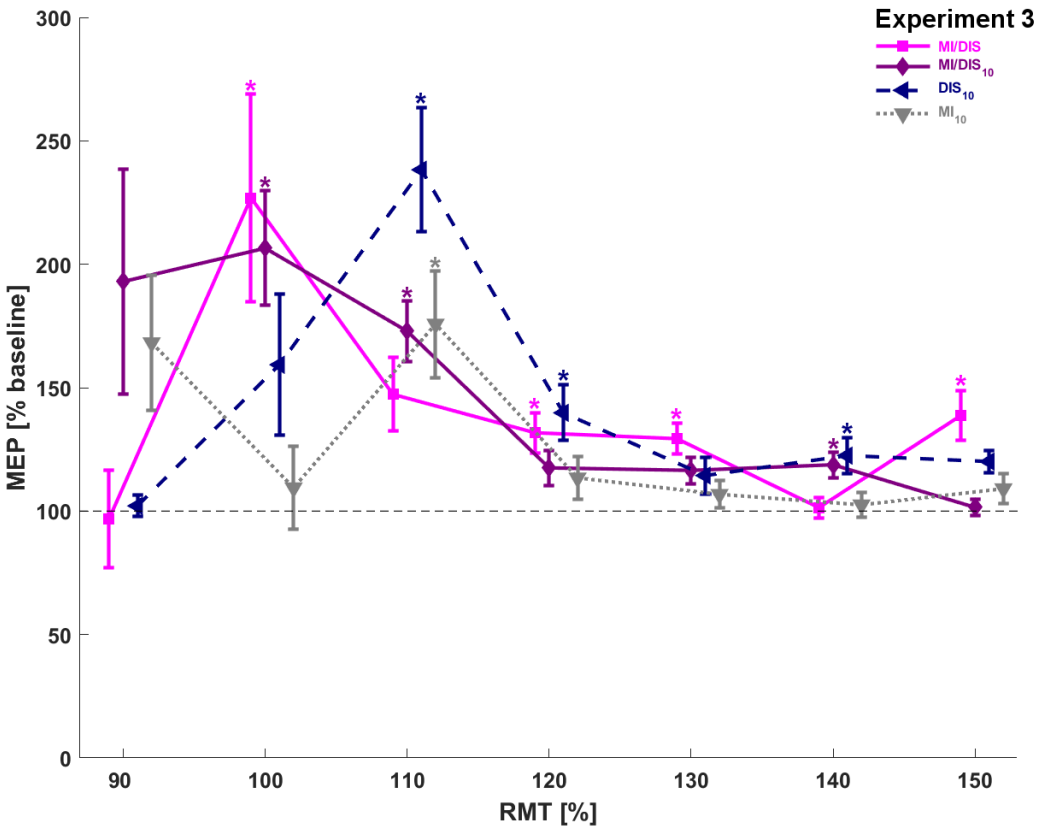

Experiment 3
MI/DIS
MI/DIS10
DIS10
MI10
MEP [% baseline]
RMT [%]
300
250
200
150
100
50
0
90
100
110
120
130
140
150

Figure 7:

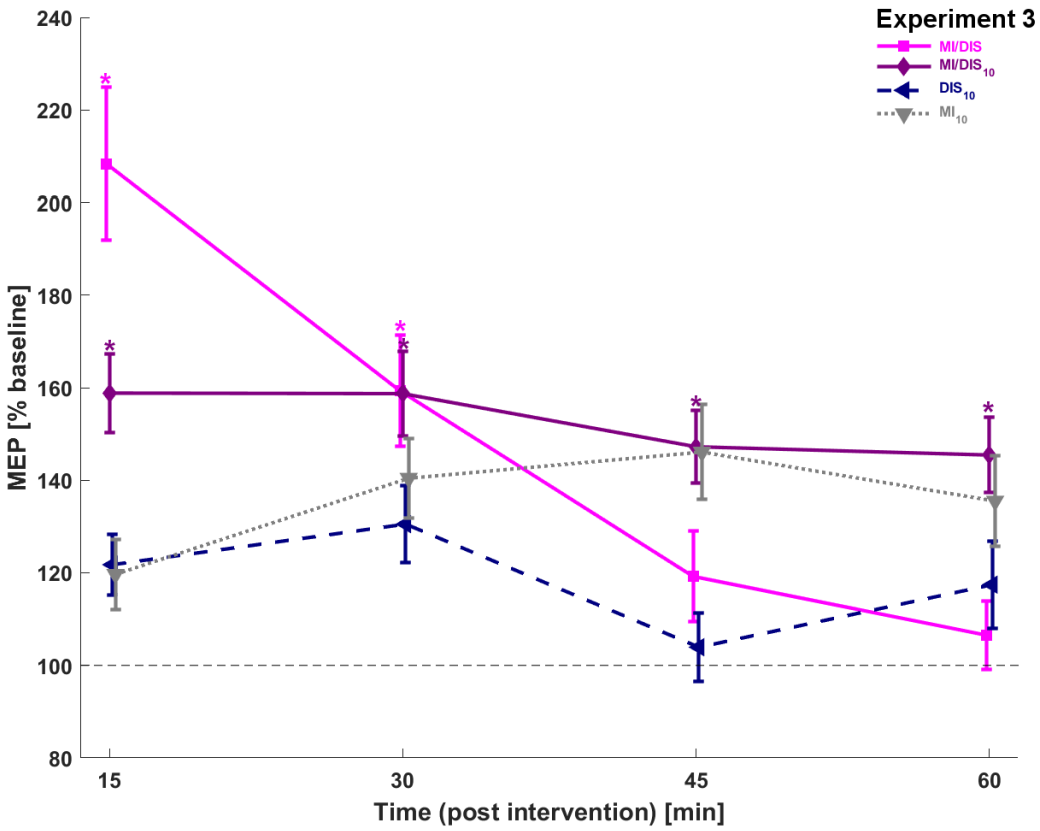
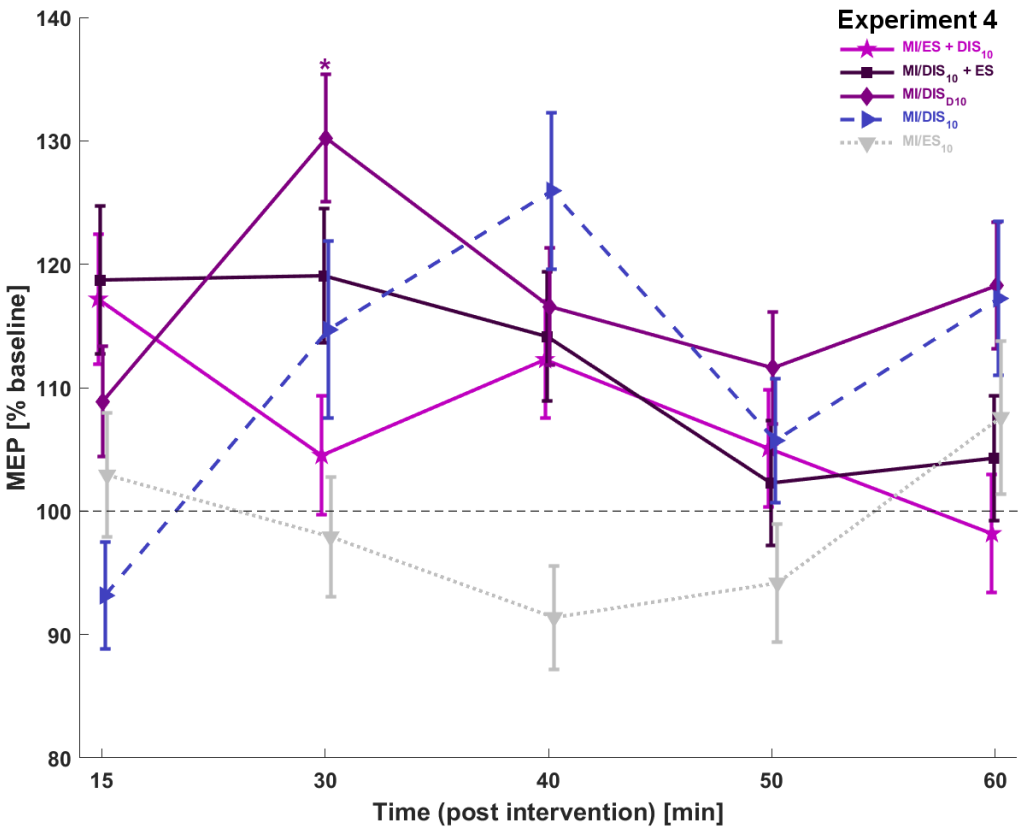

Experiment 3
MI/DIS
MI/DIS10
DIS10
MI10
MEP [% baseline]
240
220
200
180
160
140
120
100
80
15
30
45
60
Time (post intervention) [min]

Figure 8:


Experiment 4
MI/ES + DIS10
MI/DIS10 + ES
MI/DISD10
MI/DIS10
MI/ES10
MEP [% baseline]
Time (post intervention) [min]
140
130
120
110
100
90
80
15
30
40
50
60
*

8.2 Detection and enhancement of ipsilateral corticospinal connections in the human brain

Title

Detection and enhancement of ipsilateral corticospinal connections in the human brain

Short title

Detection and enhancement of ipsilateral connections

Authors

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Author contributions

L.Z. and A.G. designed research; L.Z., B.T. and A.M. performed research; L.Z., B.T. and A.G. analyzed data; L.Z. and A.G. wrote the manuscript.

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Abstract

The ipsilateral corticospinal tract (iCST) has been implied in the control of limb function and shown to adaptively reorganize if the contralateral tract is affected such as in stroke. However, as the presence of ipsilateral motor evoked potentials (iMEPs) in hand muscles has escaped recognition limited data is available. In the first part of this study, we investigated the detection of iCST for the extensor digitorum communis (EDC). Next, we aimed to enhance the iCST by combining motor imagery with a disinhibition protocol, reported previously to be efficient in potentiating the contralateral CST. We show that iMEPs can be measured reliable after TMS during BB contraction with a coil orientation of 45° to the sagittal line. These ipsilateral responses can be modified by paired-pulses transcranial magnetic stimulation (TMS) like contralateral responses, resembling short intracortical facilitation (SICF). Application of a disinhibition protocol resulted in a significant increase of the iMEPs in a muscle-specific way. We were able to show a reliable detection and significant enhancement of ipsilateral connections. This represents a new effective intervention targeting the iCST and thereby provides a therapeutic backdoor in severely affected stroke patients via the contralesional hemisphere.

Significance statement

The presence of ipsilateral muscle evoked potentials (iMEPs) did not gain enough attention. Due to missing data about intracortical properties and reliability of measurement, intervention studies targeting to modulate the plasticity of the ipsilateral corticospinal tract (iCST) are lacking crucial information. Furthermore, it remains still unclear if the iCST represents a substitute for enhancement of motor control in affected patients.

In this study with healthy subjects, new data is presented. First, we show that iMEPs are detectable in the extensor digitorum communis (EDC) with a good reliability. Based on these findings, we developed a protocol to enhance the iCST. These findings, thereby, provide necessary information about the iCST and a design for neurorehabilitation.

Introduction

The ipsilateral motor cortex (iM1) has some degree of control over the muscles of the ipsilateral limbs and has been implicated in the control of upper limb function (Liepert et al., 2001; Stinear et al., 2001; Alkadhi et al., 2002; Stippich et al., 2007; Uehara et al., 2011; Bradnam et al., 2013b; Tazoe and Perez, 2014; Vukelić et al., 2014). Detailed understanding of the ipsilateral corticospinal excitability (iCSE) is of high importance as stroke patients showed adaptive, functionally relevant cortical reorganization in the contralesional hemisphere (Calautti et al., 2007; Riecker et al., 2010; Ramos-Murguialday et al., 2013). In detail, in severely affected patients with strong damage to the ipsilesional M1, contralesional areas have to be recruited to take over the lost function (Nowak et al., 2009; Riecker et al., 2010; Small et al., 2013; Grefkes and Ward, 2014; Sankarasubramanian et al., 2017; McCambridge et al., 2018).

Transcranial magnetic stimulation (TMS) can be used to study CSE. Muscle preactivation and high stimulation intensities are required to study the properties of iCSE (Ziemann et al., 1999; Tazoe and Perez, 2014; McCambridge et al., 2016). Multiple factors influence iCSE, such as the type of upper-limb movement (Tanji et al., 1988; Cisek et al., 2003) and the muscle being investigated due to different connectivity

(Pandya and Vignolo, 1971; Jenny, 1979; Gould et al., 1986). Furthermore, changes in iM1 due to movement or motor imagery (MI) of an ipsilateral muscle have been linked to intracortical neuronal population and interhemispheric inhibition (IHI) (Liang et al., 2008; Perez and Cohen, 2008a). It was suggested that cM1 drives the activity of iM1 most likely via an interaction between IHI and intracortical inhibition (ICI) (Sanger et al., 2001; Carson, 2005), i.e., a decrease in ICI leads to an increase of IHI, and vice versa (Daskalakis et al., 2002; Lee et al., 2007). IHI is mediated via transcallosal glutamatergic pathways linking with pyramidal tract neurons through gamma-Aminobutyric acid (GABA)-ergic interneurons (Reis et al., 2008). Different disinhibition protocols targeting GABAergic interneuron networks have been shown to induce plasticity in the corticospinal tract (CST) and thereby alter CSE, e.g., MI (Takemi et al., 2013; Avanzino et al., 2015) and a repetitive paired-pulse TMS paradigm, termed disinhibition stimulation (DIS) (Cash et al., 2016). On the one side, a combination of the different disinhibition protocols may act in a synergistic way facilitating iCSE, i.e., an increased IHI directly suppresses the excitability of cortical output neurons and simultaneously makes a release of intracortical inhibition which indirectly enhances the excitability of cortical output neurons (Daskalakis et al., 2002; Liang et al., 2008). On the other hand, the combination may lead to a disruption of the modulation and result in an abolishment of the plasticity inducing mechanisms, triggered by each protocol separately. Furthermore, the differences between the combined disinhibition and a separate disinhibition protocol might reveal new insights into the interplay of intracortical circuits and interhemispheric inhibition.

In the current study we focused on a wrist extensor, e.g., the extensor digitorum communis (EDC) muscle, as existing data is limited and showed only inconsistent responses (Ziemann et al., 1999; Alagona et al., 2001). Furthermore, the EDC muscle activity is essentially affected after stroke and therefore an important muscle for studies investigating protocols that may be used for neurorehabilitation.

The purpose of this study was to measure consistent and reliable responses in the targeted muscle and understand the underlying physiology, e.g., intracortical connections. After refining the detection and gaining new knowledge about interneuronal networks, we tested a combination of disinhibition protocols, e.g., MI and DIS. We hypothesized that MI led to a general release of ICI and thus will further enhance the plasticity-inducing potential of DIS for the ipsilateral CST.

Materials and methods

Study design

A total of 78 healthy subjects (mean age 24.5 ± 3.1 years, range 19-33 years, 48 females) participated in this study, which consisted of a pre-study (Experiments 1-5) and the main study Experiment 6 (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. Experiments were separated by at least 2 weeks and evolved in their design based on the findings in the previous experiments in order to reliable detect and finally enhance iCSE. In Experiment 6, three conditions were investigated in a blinded study design with randomized order and separated by at least two days to avoid carry-over effects. Subjects were not informed as to the purpose and hypothesis of each experiment. All sessions of the main study were conducted at a similar time of day to minimize the effect of circadian fluctuations due to cortisol on CSE (Sale et al.,

2008). The subjects had no contraindications to TMS (Rossi et al., 2009) and no history of psychiatric or neurological disease. Right-handedness was confirmed by the Edinburgh handedness inventory (Oldfield, 1971).

The number of subjects in each experiment was as follows: Experiment 1: 15 subjects; Experiment 2: 9 subjects; Experiment 3: 21 subjects; Experiment 4: 20 subjects; Experiment 5: 28 subjects; Experiment 6: 20 subjects. One subject participated in Experiment 1-4; one subject participated in Experiment 1-6; two subjects participated in Experiment 1-2; one subject participated in Experiment 1, 5 and 6; one subject participated in Experiment 3-6; three subjects participated in Experiment 4-6.

The purpose of Experiment 1 was to test for connections in the ipsilateral extensor digitorum communis (iEDC) muscle using 4 different coil orientations (45°, 135°, 225°, and 315° to the sagittal plane).

The follow-up Experiment 2 consisted of two different muscles being contracted extensor digitorum communis (EDC) or biceps brachii (BB) at four different contraction levels (30, 50, 70, and 90% of maximum voluntary contraction (MVC)). This adjustment was performed as an iEDC preactivation at 30% MVC resulted only in a low ipsilateral muscle evoked potential (iMEP) probability in Experiment 1.

The follow-up Experiment 3 analyzed the influence of the stimulation point, i.e., contralateral hotspot vs. ipsilateral hotspot.

In the follow-up Experiment 4 the influence of different IPIs (1.3, 1.5, 2, 3, 4.5, 150, 200, 220, 250, and 300ms) on iCSE was measured to test for intracortical properties.

In the follow-up Experiment 5 the intra-subject retest reliability was tested for iCSE on three experimental days separated by at least two days to avoid carry-over effects.

In the main study Experiment 6 we designed a protocol based on previous findings to enhance iCSE. Experiment 6 consisted of three conditions (MI/DIS, DIS, MI): A DIS protocol (Cash et al., 2016; details see below) was paired with MI of finger and wrist extension (Kraus et al., 2016a; details see below). Five stimulation doublets (i.e.,, ten stimuli) were applied at the time of imagined movement (during the MI phase) in four runs each fifteen trials (MI/DIS condition). Altogether, 480 stimuli were applied during 60 trials. To test for associativity of the intervention, the same DIS protocol was applied while performing a mental calculation task (iterated multiplications by 2) (Kanthack et al., 2017), i.e.,, *without* a motor imagery task (DIS condition). This was expected to control for attention distraction effects. Moreover, the 60 MI trials were performed with the TMS coil tilted 90° off the head with both wings touching the skull at the motor hotspot since it is unlikely to evoke any biological effects with this sham procedure (MI condition) (Lisanby et al., 2001).

The material and methods of data acquisition applied here were identical to those of previous studies (Kraus et al., 2016b, 2016a) and are cited accordingly:

Data Acquisition

Electromyography (EMG) data were recorded (BrainAmp Amplifier) at a sampling rate of 5kHz using an antialiasing band-pass filter with cutoff frequencies at 0.16Hz and 1kHz (Kraus et al., 2016a). Impedances at all electrodes were kept below 10kΩ. In a next step, data were transferred for online analysis to MATLAB, where they were stored for offline analysis (Kraus et al., 2016a).

Ag/AgCI AmbuNeuroline 720 wet gel surface electrodes (Ambu GmbH, Germany) were used to record electromyography (EMG) activity from the ipsilateral and contralateral EDC (iEDC and cEDC, respectively), the ipsilateral BB (iBB), and the ipsilateral flexor carpi radialis (iFCR) muscles. Two electrodes were placed on the muscle belly 2cm apart from each other.

TMS protocol

A biphasic TMS pulse (MagPro-R30 + MagOption MagVenture, Germany) was applied through a figure-of-eight coil (MCF-B70). If not specified otherwise an orientation of 45° to the sagittal plane was chosen so that the induced current was directed posterior to anterior in the first phase, and anterior to posterior for the second phase of the stimulus. Frameless stereotactic neuro-navigation (TMS Navigator, Localite GmbH, Germany) supported the localization of the TMS target position. Once a template MRI (MNI ICBM152 non-linear symmetric T1 Average Brain) had been registered to the head of each participant, the neuro-navigation system tracked the relative positions of the TMS coil and the participant's head during the experiment whilst keeping the stimulation location constant (Mathew et al., 2016). Subjects were seated comfortably in an armchair with their elbows semiflexed; the forearm was pronated, fully relaxed, and supported by the arm of the chair. The representation of the cEDC in the left M1 was determined for each subject prior to the onset of the experimental session (Kraus and Gharabaghi, 2015, 2016). We used 40% MSO as initial intensity and the anatomically defined 'hand knob' of M1. Whenever the initial stimulator output did not suffice to elicit MEPs, we increased the output in steps of 5%. We ensured that the orientation of the coil remained perpendicular to the central sulcus and 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 (Groppa et al., 2012).

For Experiment 1, 10 TMS pulses were delivered at 90% MSO during iEDC contraction at 30% MVC with a coil orientation of 45°, 135°, 225°, and 315° to the sagittal plane.

For Experiment 2, 10 TMS pulses were delivered at 90% MSO during EDC or BB contraction. The MVC was 30, 50, 70, or 90% in randomized order.

In the following Experiments 3-6, we tested for iMEPs with a BB contraction at 30% MVC. This change was performed, because BB contraction resulted in a better iMEP probability in the iEDC (see in Results).

In Experiment 3, a cortical map representation was acquired. A 5-by-5 grid (1 x 1 cm per cell) was predefined in the navigation software. 5 TMS stimuli at 90% MSO were applied at each grid cell in a randomized order. Next, the contralateral (cHS) and resulting ipsilateral (iHS) hotspot was analyzed using 10 TMS stimuli at 50, 60, 70, 80, 90, or 100% MSO in a randomized order.

For Experiment 4, 10 conditioning stimuli (CS) and 10 paired-pulse TMS with an IPI of 1.3, 1.5, 2, 3, 4.5, 150, 200, 220, 250, and 300 ms was tested in a randomized order.

In Experiment 5, subjects were measured on three different days with an inter-test duration of at least 48h. 20 TMS pulses were applied at 90% MSO.

For Experiment 6, we by applied 20 TMS pulses at 90% MSO to determine iCSE at baseline (prior to intervention) and after the intervention. In detail, post intervention measures were performed 15 and 30 min after the intervention.

Disinhibition stimulation (DIS)

In Experiment 6, cortical stimulation was performed on the basis of a paired-pulse rTMS protocol, referred to as disinhibition stimulation (DIS) in this manuscript for the sake of simplicity and to remain consistent with previous literature applying the same approach (Cash et al., 2016). DIS consisted of a train of five biphasic TMS doublets (ten pulses) at 110% RMT. The applied interpulse interval (IPI) of a doublet as well as the interdoublet interval (IDI) to induce SICF and LCD, respectively, were individually adjusted to enhance the impact of DIS on iCSE (Sewerin et al., 2011). Specifically, IPIs of 1.3, 1.5, and 2 ms, and IDIs of 200, 250, and 300 ms were investigated for every subject in the beginning of each experiment, based on findings of pre-study experiments.

Motor imagery (MI)

In Experiment 6, each intervention consisted of four runs with fifteen trials each. 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 was 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 focusing on the sensory information, and to relax during the other periods (Gharabaghi et al., 2014; Bauer et al., 2015; Gharabaghi, 2015; Vukelić and Gharabaghi, 2015a; Royter and Gharabaghi, 2016). DIS was initiated 3 s after the 'go' cue during the MI period, since this was the time when the strongest ERD was detected in our previous work (Vukelić et al., 2014; Bauer et al., 2015, 2016; Gharabaghi, 2015; Vukelić and Gharabaghi, 2015a, 2015b; Royter and Gharabaghi, 2016).

Data processing

For iCSE (Figure 1), data was analyzed from rectified EMG, comparing the post-stimulus EMG activity to the background (BG) EMG activity 100 ms before the stimulus. ΔiMEPs (μV ms) were measured using the following equation:

$$\Delta iMEP = \left(iMEP_{amplitude} - BG\right) * iMEP_{duration}$$

where $iMEP_{duration}$ is the period in which the poststimulus EMG exceeds the BG mean ± one standard deviation (SD) (Ziemann et al., 1999). Probability of iMEPs was calculated as the occurrence of an iMEP above the BG mean ± one SD for at least 5 ms on a single trial basis. Latency was the earliest deflection of the EMG that was maintained above the BG mean ± one SD for at least 5 ms. For cCSE, the EMG data was inspected during offline analysis, discarding any trials containing muscle preactivation (rectified prestimulus EMG activity above 20 μV). Less than 5% of all trials were rejected due to contamination by muscle activity. The artifact-free cMEP amplitudes were then measured peak to peak. Latency of cMEP was the earliest deflection of the EMG above the BG mean ± one SD. Data were analyzed offline using custom written scripts in MATLAB (R2017b, The MathWorks, Inc., United States).

Statistical analysis

Statistical analysis was performed using statistical functions in MATLAB (R2017b, The MathWorks, Inc., United States) and the *psych* package (Revelle, 2017) in R (version 3.4.3).

Data sets undergoing analysis of variance (ANOVA) were assessed for equality of variances using Levene's test, and if necessary, log-transformed. An ANOVA with random effect *subject* and the below mentioned parameters was used to assess changes. To investigate optimal coil orientation, the fixed factor *orientation* and the dependent variable *iMEP probability* were used. To investigate muscle being contracted, the fixed factor $muscle_{contracted}$ and the dependent variable *iMEP probability* were used. To investigate the different stimulation points, the fixed factor *point of stimulation* and the dependent variable *ΔiMEP* were used. To investigate MVC effects, the fixed factors *MVC* and the dependent variable *ΔiMEP* were used. To investigate optimal IPI, the fixed factors *IPI* and the dependent variable *ΔiMEP* were used. To investigate differences in latency, the fixed factors *muscle* and the dependent variable *latency* were used. To investigate intervention effects on iCSE or cCSE, the fixed factors *condition* and *time*, and the dependent variable *ΔiMEP* were used. If a significant effect was detected and the factor had multiple dimensions, post hoc tests were performed using Tukey's test.

To test the retest reliability an intraclass correlation coefficients (ICC) test was run. ICC estimates were calculated based on a two-way random effects, absolute agreement, multiple measurements setup, e.g., ICC(2,3) (McGraw and Wong, 1996). Values less than 0.5 are indicative of poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values greater than 0.9 indicate excellent reliability (Fleiss and Cohen, 1973).

The alpha level was set at $p \leq 0.05$. Results are expressed as mean ± 95% confidence interval, unless stated otherwise.

Results

Detection of iCSE

No significant effect of coil orientation on ΔiMEP was detectable (Figure 2A; $F_{3,63}=1.71$, $p=0.178$). The iMEP probability was significantly increased with the BB being contracted (Figure 2B; $F_{1,179}=6.43$, $p=0.012$). No significant effect of stimulation point on the ΔiMEP was detectable (Figure 2D; $F_{1,2519}=0.06$, $p=0.801$). The retest reliability of the ΔiMEP showed an ICC(2,3) of 0.81 (Figure 3A, $F_{27,54}=5.08$, $p<0.001$). The retest reliability for the cMEP of the right EDC had an ICC(2,3) of 0.81 ($F_{27,54}=5.14$, $p<0.001$). No significant effect of MVC on ΔiMEP was detectable (Figure 3B; $F_{3,35}=0.39$, $p=0.760$). The comparison of ΔiMEP normalized to the CS, ANOVA revealed a significant effect of IPI (Figure 3C, D; $F_{10,2199}=5.14$, $p<0.001$). Post hoc analysis showed a significant increase of ΔiMEP at an IPI of 1.3 ms compared to CS ($p=0.029$; Tukey's test) and 220 ms compared to 150 ms and 300 ms ($p=0.016$; Tukey's test). The latency of the iMEP (27.12 ± 0.66) lagged significantly behind the latency of the cMEP in the cEDC (23.97 ± 0.32) ($F_{1,66}=4.02$; $p<0.001$).

Enhancement of iCSE

For the iEDC, ANOVA revealed a significant effect of time and condition interaction on the ΔiMEP (Figure 4A; effect of time: $F_{2,3599}=2.64$, $p=0.072$; effect of condition: $F_{2,3599}=2.65$, $p=0.071$; effect of interaction: $F_{4,3599}=3.87$, $p=0.004$). DIS significantly

increased the ΔiMEP at post 30 to 145.6 ± 9.2 % of baseline (p=0.014; Tukey's test), to 41.1 ± 9.2 % of MI/DIS (p=0.041; Tukey's test), and to 56.5 ± 9.2 % of MI (p<0.001; Tukey's test).

For the iFCR, no significant interaction was detected (Figure 4B; effect of time: $F_{2,3599}$=1.36, p=0.953; effect of condition: $F_{2,3599}$=1.36, p=0.266; effect of interaction: $F_{4,3599}$=0.98, p=0.418).

For the cEDC, there was a significant effect of time, condition, and the interaction (Figure 4C; effect of time: $F_{2,2906}$=6.90, p=0.001; effect of condition: $F_{2,2906}$=41.05, p<0.001; effect of interaction: $F_{4,2906}$=15.32, p<0.001). DIS significantly increased the ΔiMEP at post 15 and 30 up to 124.6 ± 2.2 % of baseline (p=0.001; Tukey's test), up to 127.4 ± 2.2 % of MI/DIS (p<0.001; Tukey's test), and to 132.0 ± 2.2 % of MI (p<0.001; Tukey's test).

Discussion

In this study with healthy subjects, we investigated the optimal protocol to elicit reliable iMEPs in the EDC muscle, a muscle highly affected in stroke patients and previously not investigated in detail. Furthermore, we tested a repetitive paired-pulse TMS protocol (DIS) with the aim of modulating the iCSE and were able to induce muscle-specific changes.

Detection of ipsilateral connections

The optimal protocol for eliciting iMEPs in the EDC was to choose the contralateral hotspot and stimulate at a 45° coil orientation at 90% MSO with the BB being contracted at 30% MVC. This is in contrast to previous studies which showed iMEPs had a different preferred current direction for activation when compared with cMEPs (Ziemann et al., 1999). In general have studies of ipsilateral projections been equivocal, as some showed no difference in the spatial location of the ipsilateral and contralateral center of gravity (MacKinnon et al., 2004) and others showing a medial–posterior shift in the ipsilateral location (Ziemann et al., 1999; Alagona et al., 2001). The observed differences to previous studies might be due to the different muscles being investigated, as proximal and distal arm muscle representations differ in morphological and functional differences, e.g., transcallosal projections (Pandya and Vignolo, 1971; Jenny, 1979; Gould et al., 1986). Another interesting finding was the increased iMEP probability for the EDC muscle, when the BB and not the EDC was preactivated. A possible explanation for this finding is the dependence of the activity in ipsilateral pyramidal neurons on the type of upper-limb movement (Tanji et al., 1988; Cisek et al., 2003). These findings further underline the importance of characterizing the muscle of interest in detail before investigating potential changes in the iCSE due to a plasticity enhancing intervention.

Retest reliability

The reliability of the assessment tool is an important criterion to ensure that changes observed are in relation to physiological changes and not to chance. The inter-session reliability of iMEP measurements was good and similar to the one of cMEPs (Bastani and Jaberzadeh, 2012; Goldsworthy et al., 2016; Kraus and Gharabaghi, 2016), which are accepted as a reliable outcome measure to characterize properties of the contralateral corticospinal tract (cCST) (Di Lazzaro et al., 2004, 2012; Ziemann et al., 2015).

Ipsilateral pathways

Although most corticospinal fibers decussate to form the cCST, ipsilateral pathways forming the iCST exist (Gerloff et al., 1998; Brus-Ramer et al., 2009; Bradnam et al., 2013a; Wahl et al., 2017). The latency of the iMEP we measured in the iEDC (27.12 ± 0.66) differed significantly from the latency of the cMEP in the cEDC (23.97 ± 0.32). The differences reported here may be slightly underestimating the delay, as activation of ipsilateral muscle slightly shortened the latencies by approximately 1 ms (Alagona et al., 2001). Nevertheless, the observed latency difference is similar to the lag described in the literature (Ziemann et al., 1999; Bradnam et al., 2010; Tazoe and Perez, 2014; McCambridge et al., 2016) and points towards the involvement of an oligosynaptic pathway or a longer conduction distance from the excited cortical area to the origin of its descending pathway. These descending pathways may originate in the medial brainstem and include the reticulospinal pathway (Riddle et al., 2009; Baker and Perez, 2017) and the rubrospinal pathway (Ishida et al., 2016). Another possibility are midline crossing fibers of the cCST (Starkey et al., 2012; Wahl et al., 2017). It is unlikely that the ipsilateral responses have been affected by the opposite hemisphere, as transcallosal activation requires approximately 10-20 ms (Cracco et al., 1989; Meyer et al., 1998) and is therefore too long to account for the delays we found between the onset of the cMEP and the iMEP. However, as the presence of iMEPs in hand muscles in response to TMS has escaped recognition due to the high threshold for activation and required voluntary muscle contraction (Wassermann et al., 1994; Ziemann et al., 1999), not enough data is available in the literature for a detailed statement about the investigated ipsilateral pathway.

Intracortical properties

Paired pulse TMS enables the assessment of intracortical excitatory and inhibitory circuits (Kujirai et al., 1993; Ziemann et al., 1996). A suprathreshold TMS of M1 evokes multiple descending volleys (I-waves) at a periodicity of about 1.5 ms from transsynaptic activation of principal cells through excitatory interneuronal connections (Di Lazzaro et al., 2012). An initial conditioning stimulus activates populations of interneurons, which in turn cause time-dependent modulation of the MEP response to the subsequent test stimulus and allows the investigation of intracortical properties, e.g., SICF (Schwerin et al., 2011) and LCD (Cash et al., 2010). Previous work has shown that it is possible to target inhibitory networks in the iCST (McCambridge et al., 2016). However, to our knowledge, we are the first to show that SICF and LCD can also be measured for the iCST. As the facilitation is dependent on I-wave generating neurons (Ziemann et al., 1998; Ilić et al., 2002; Thickbroom, 2011), we can assume that the ipsilateral pathway is modulated by I-wave generating neurons. In comparison to previous studies (McCambridge et al., 2016), we were not able to detect an inhibitory effect. However, studies for the cCST showed a relationship between the degree of ICI and CS intensity as an inverted U-shaped curve (Chen et al., 1998; Ilić et al., 2002) and the high intensity used for CS in our protocol (90% MSO) may have led to reduction of inhibition and subsequent facilitation. Additionally, the time course of inhibitory and facilitatory effects varies in a task-dependent manner (Caux-Dedeystère et al., 2014), an could therefore have been modified by the BB preactivation. It remains unclear if the facilitation is mediated in the exact same way as for the cCST, e.g., activation of glutamatergic interneurons for SICF (Ziemann et al., 1998; Ilić et al., 2002) and $GABA_B$ mediated disinhibition for LCD (Cash et al., 2011).

Enhancement of ipsilateral connections

DIS stimulation alone, but not in combination with MI, resulted in a significant increase of iCSE up to 145.6 ± 9.2 % of the baseline. MI leads to an event-related desynchronization (ERD) and activates muscle specific excitatory neurons (Takemi et al., 2013). Bilateral ERD during movement or its imagination has been shown (Vukelić et al., 2014; Hasegawa et al., 2017). However, MI also results in an IHI (Liang et al., 2014) and might thereby modulate the plasticity inducing effect of DIS, as ICI and IHI affect each other in a negative feedback loop (Daskalakis et al., 2002; Lee et al., 2007). IHI involves activity across the corpus callosum through dense projections (Ferbert et al., 1992; Meyer et al., 1995) and is mediated by a decrease of the last I-wave (Di Lazzaro et al., 1999). Additionally, an involvement of transcallosal glutamatergic pathways linking with pyramidal tract neurons through $GABA_B$-mediated inhibitory neurons has been proposed (Ferbert et al., 1992; Reis et al., 2008; Perez and Cohen, 2009). In this study, we analyzed the combination of two disinhibition protocols, each targeting GABAergic interneuron networks. MI acts via both $GABA_A$ and $GABA_B$ receptors (Abbruzzese et al., 1999; Stinear and Byblow, 2004; Takemi et al., 2013; Chong and Stinear, 2017), whereas DIS targets $GABA_B$ receptors (Cash et al., 2016). IHI might have suppressed the excitability of cortical output neurons stronger than it disinhibited the intracortical neuronal population. Due to the modulation of $GABA_B$ergic neurons by MI-induced IHI, it is likely that the efficiency of DIS was inhibited. This hypothesis is further supported by the suppression of effects for the cCST due to inhibition of disinhibition by IHI.

Limitations and future perspectives

A limitation of this study is that we studied only healthy subjects. Therefore, it remains unclear if the same properties of the iCST and the high inter-session reliability can be observed in affected patients, e.g., stroke patients. Furthermore, we tested only the reliability between session and not within a session. Future studies need to investigate the intra-reliability of iCSE measurements.

A bypass of the negative effect of IHI might be the up-regulation of the targeted hemisphere by contraction of the contralateral hand with or without MI of the ipsilateral side. In detail, through an activation of the contralateral side a decrease of IHI from the opposite cortex (Liang et al., 2011) and an disinhibition of the intracortical circuits (Muellbacher et al., 2000; Perez and Cohen, 2008b) may be induced, while still facilitating iCSE. However, this hypothesis requires a more detailed understanding of the interaction between IHI and MI on iCSE and therefore further investigation.

Conclusion

In conclusion, iMEPs can be detected in the EDC muscle with a good reliability and be modulated by intracortical circuits. DIS was able to modulate iCSE and is therefore a powerful tool for plasticity induction. These findings may thus provide a therapeutic backdoor using interventions to modulate the ipsilateral pathway and the pressing into service of normally inhibited routes when contralateral connections are no longer a viable target for plasticity enhancing interventions, e.g., in severely affected stroke patients.

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Figures

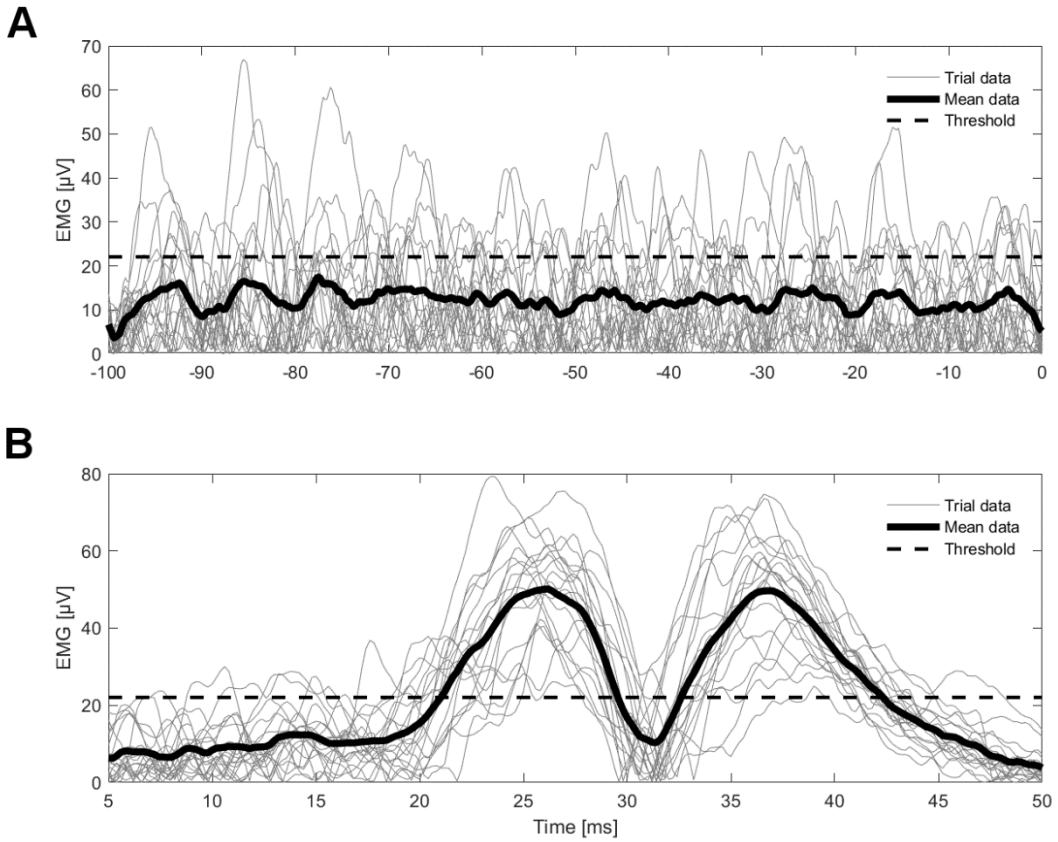

Figure 1: Rectified electromyography (EMG) recordings from the ipsilateral EDC (iEDC) of a representative participant. The averaged trace is shown in black, single trial data is shown in gray. The threshold for detection was the mean EMG of background (BG) + one standard deviation (SD) of BG

A: BG activity of the iEDC during contraction of the BB.

B: EMG from iEDC after TMS. Ipsilateral MEPs were measured as ($iMEP_{amplitude}$ - BG)*$iMEP_{duration}$.

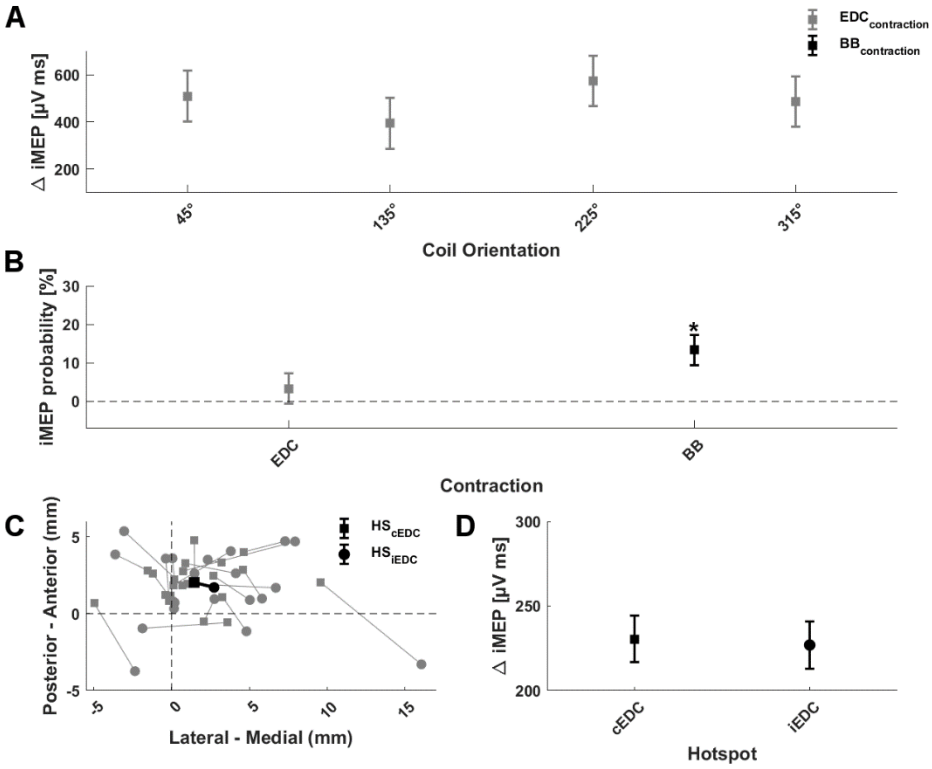

Figure 2: Possible parameters influencing the detection of iCSE during EDC contraction (grey) or BB contraction (black)

A: ΔiMEP for different coil orientation during iEDC contraction. No significant differences were detectable.

B: iMEP probability significantly increased during BB contraction compared to EDC contraction (* indicates $p < 0.05$, Tukey's test).

C: Comparison of the centres of gravity (CoG) of the maps for the ipsilateral (circles) and the contralateral MEP (squares) in 21 subjects. The black symbols show the means of the CoG across subjects. No clear shift was detected. The origin of the cartesian coordinate system refers to the coil location, which was determined during the hotspot search for cEDC in the beginning.

D: ΔiMEP did not differ significantly between the contralateral and ipsilateral stimulation hotspots.

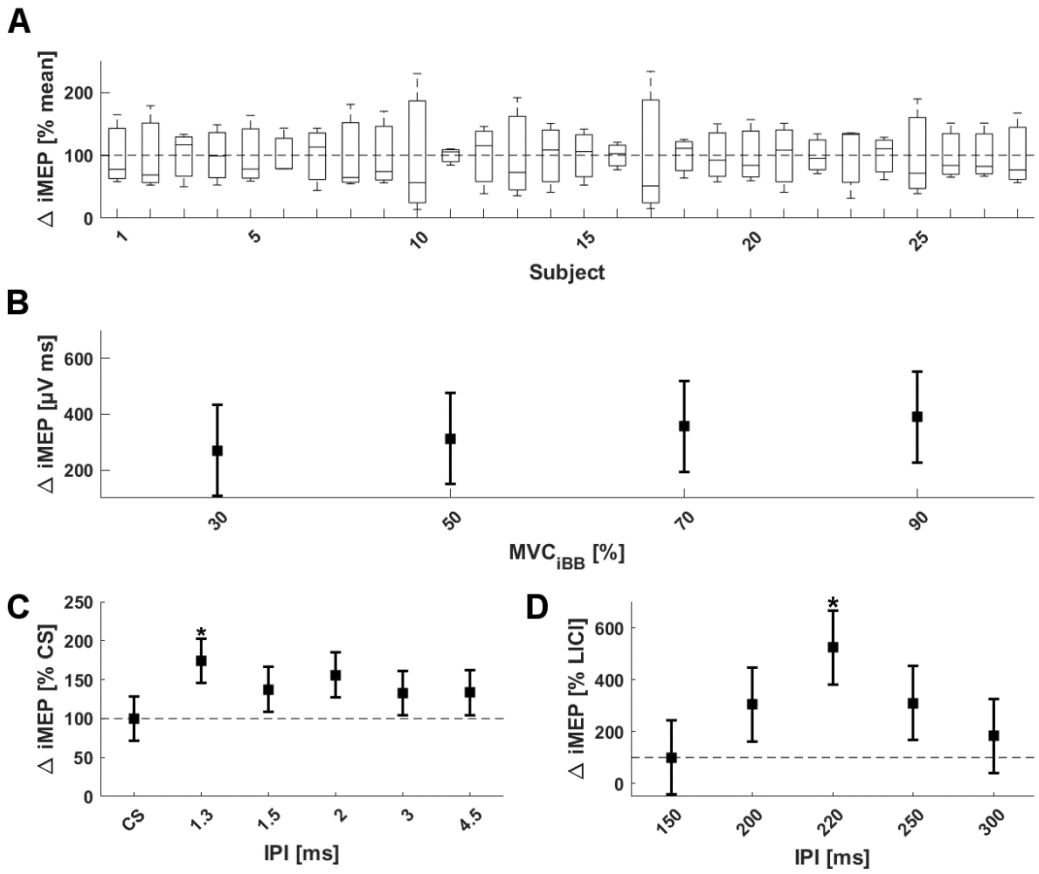

Figure 3: Modulation and reliability of ΔiMEP

A: ΔiMEP retest reliability showed an ICC(2,3) of 0.81 ($p<0.001$). Data is shown normalized to the interindividual mean of all three test sessions.

B: ΔiMEP did not differ significantly between different contraction forces.

C: Changes of ΔiMEP after paired-pulse TMS normalized to CS. An IPI of 1.3 ms significantly increased the ΔiMEP (* indicates $p < 0.05$, Tukey's test).

D: Changes of ΔiMEP after paired-pulse TMS normalized to LICI at 150 ms. An IPI of 220 ms significantly increased the ΔiMEP (* indicates $p < 0.05$, Tukey's test).

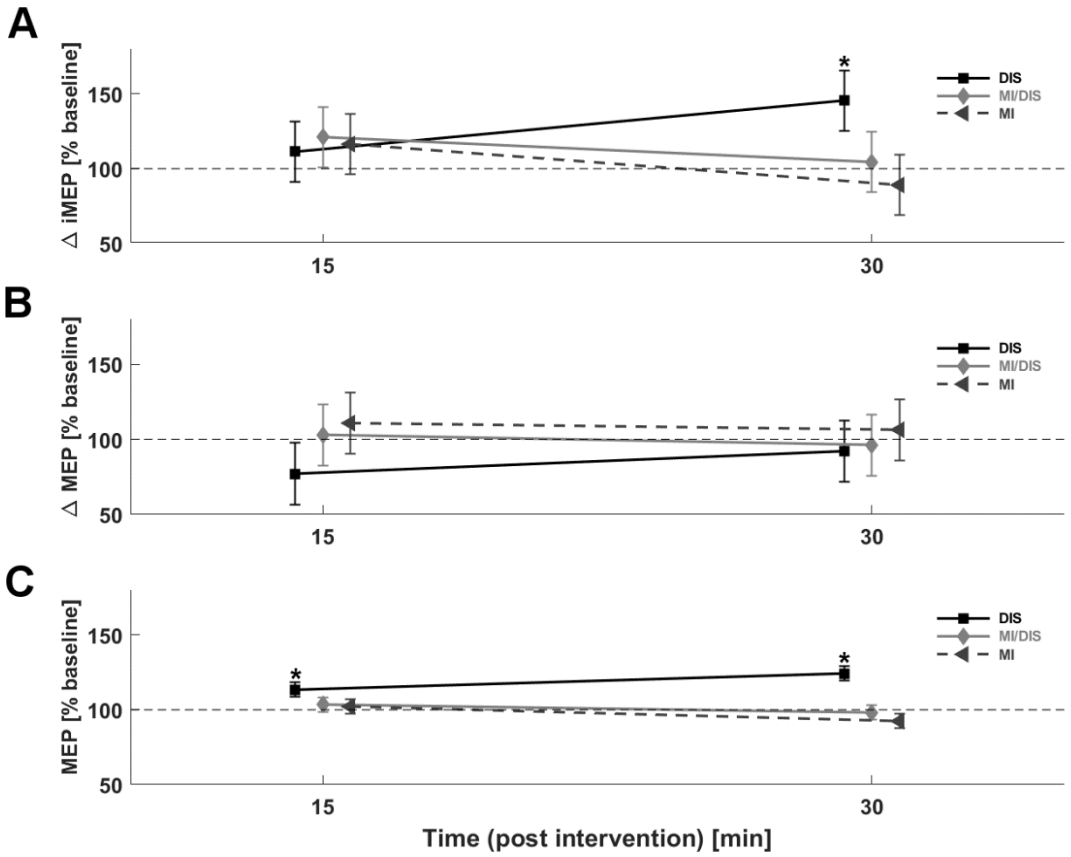

Figure 4. *Time-course of ΔiMEP changes post intervention for iEDC (A) and iFCR(B), and of MEP changes post intervention for cEDC (C).*

A: DIS showed a significant increase of iCSE for the iEDC 30 min after the intervention (* indicates $p < 0.05$, Tukey's test).

B: No changes were observed for the iFCR.

C: DIS showed a significant and consistent increase of MEPs for the cEDC after the intervention (* indicates $p < 0.05$, Tukey's test).

8.3 Motor task dependent modulation of disinhibition stimulation to enhance ipsilateral corticospinal connections for stroke rehabilitation

Title

Motor task dependent modulation of disinhibition stimulation to enhance ipsilateral corticospinal connections for stroke rehabilitation

Short title

Task dependent enhancement of ipsilateral connections for stroke rehabilitation

Authors

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Author contributions:

L.Z. and A.G. designed research; L.Z., B.T. and P.K. performed research; L.Z., B.T. and A.G. analyzed data; L.Z. and A.G. wrote the manuscript.

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Abstract

Multi-modal neurorehabilitation models suggest targeting the contralesional primary motor cortex (M1) in severely affected stroke patients. However, the efficacy of targeting ipsilateral corticospinal tract (CST) from contralesional M1 remains unclear. Here we show that the combination of motor execution with transcranial magnetic stimulation to induce disinhibition elicits significant enhancement of ipsilateral corticospinal excitability (CSE) in healthy subjects. These changes were muscle-specific and persistent. Next, we applied the disinhibition protocol in severely affected chronic stroke patients. Stroke patients exhibited a significant increase of ipsilateral CSE not observable in the control sham intervention. Results illustrate a new effective intervention targeting the ipsilateral CST and thereby provides a therapeutic backdoor in severely affected stroke patients via the contralesional hemisphere.

Significance statement

Targeting the ipsilesional primary motor cortex (M1) may not be a ‘one size fits all’ approach and multi-modal models suggested targeting the contralesional M1 in severely affected patients. However, intervention studies targeting the contralesional M1 are sparse and it remains unclear if the ipsilateral corticospinal tract (CST) represents a legitimate substitute for enhancement of motor control in severely affected patients.

In this study with both healthy subjects and chronic stroke patients, new data is presented. We show that ipsilateral corticospinal excitability can be increased in both populations using a combined protocol to target disinhibition of the contralesional M1, e.g., combination of motor execution with repetitive paired-pulse transcranial stimulation.

Introduction

Stroke remains a leading cause of long-term adult disability, and the global burden of stroke continues to grow (Feigin et al., 2015). Patient outcomes are related to initial stroke severity and functionality of contralateral corticospinal tract (CST) from ipsilesional primary motor cortex (M1) (Stinear et al., 2012). Therapeutic models and their investigation are required for patients at the chronic stage (Small et al., 2013; Plow et al., 2016).

The common approach towards design of interventions is the hypothesis that over-excitation of contralesional M1 inhibits corticospinal excitability (CSE) of ipsilesional M1 via interhemispheric inhibition (IHI) (Murase et al., 2004). Based on this premise, interventions consists of facilitation of ipsilesional M1 with concurrent inhibition of contralesional M1 (Di Lazzaro et al., 2008; Halko et al., 2011; Sung et al., 2013).

However, this may be too simplistic and unsuccessful in patients who suffered from extensive damage to ipsilesional CST (Bradnam et al., 2013; Di Pino et al., 2014; Plow et al., 2016). Some studies did not detect an asymmetry in IHI (Bütefisch et al., 2008; Stinear et al., 2015). Others failed to improve motor functions in severely affected patients after upregulate ipsilesional M1 and/or downregulate contralesional M1 (Ackerley et al., 2010; Talelli et al., 2012; Levy et al., 2016).

Based on these observations, models taking into account the extent of structural damage and availability of residual motor pathways have been proposed (Bradnam et

al., 2013; Di Pino et al., 2014; Plow et al., 2016), i.e., less affected patients rely on ipsilesional M1, while more affected patients rely on undamaged contralesional motor cortices for recovery. In detail, less affected patients have a higher structural reserve and functional contralateral CST of ipsilesional M1 may be the target of facilitating therapies (Di Lazzaro et al., 2008; Halko et al., 2011; Sung et al., 2013). However, in severely affected patients with strong damage to ipsilesional M1, contralesional areas have to be recruited to take over lost function (Nowak et al., 2009; Riecker et al., 2010; Small et al., 2013; Grefkes and Ward, 2014; Sankarasubramanian et al., 2017; McCambridge et al., 2018).

Motor recovery can be facilitated by a functional decrease of gamma-aminobutyric acid-ergic (GABAergic) inhibition (Lazar et al., 2010; Blicher et al., 2015). Unilateral hand movement was able to modulate the CSE of both motor cortices (Hortobágyi et al., 2003; Zijdewind et al., 2006; Stippich et al., 2007; Perez and Cohen, 2008; Chiou et al., 2013). This was accompanied by a decrease of intracortical inhibition (ICI) (Goodwill et al., 2012; Chiou et al., 2013) and may lead to an increased plasticity (Nudo et al., 1996; Nudo, 2003) via GABAergic modulation (Schneider et al., 2002). Another possibility to target intracortical circuits is via paired-pulse transcranial magnetic stimulation (TMS) (Kujirai et al., 1993; Ziemann et al., 1996). Combination of multiple paired-pulses modulates glutamatergic and GABAergic interneurons, resulting in a disinhibition of GABAergic driven ICI (Thickbroom et al., 2006; Cash et al., 2010). Taking together these observations, a repetitive paired-pulse TMS paradigm, referred to as DIS, was implemented and shown to increase CSE (Cash et al., 2016),

In this study, we analyzed the combination of two disinhibition protocols, each targeting GABAergic interneuron networks. DIS in combination with voluntary motor activity of non-paretic limb is a potential mechanism to release the contralesional M1 from GABAergic mediated inhibition and thus inducing plasticity not only in contralateral, but also ipsilateral CST.

We first assessed the potential of the combined disinhibition protocols in healthy subjects. After successful positive modulation of ipsilateral CSE, we tested the optimal protocol in chronic, severely affected stroke patients.

Materials and methods

Study design

A total of twenty healthy subjects (mean age 24.3 ± 3.4 years, range 20-30 years, 13 female) participated in this study, which consisted of three different experimental days (see below).

We enrolled 27 stroke patients (mean age 61.5 ± 9.4 years, range 44-77 years, 13 females) in a follow-up study, which consisted of two different interventions (see below). All patients were first-ever unilateral stroke patients.

All subjects and patients 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. For healthy subjects, three conditions were investigated in a double-blinded study design with randomized order and separated by at least two days to avoid carry-over effects. For the patients, two conditions were investigated in a blinded study design with randomized order and separated by at least one hour. Healthy subjects and patients

were unaware of 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 (Sale et al., 2008). Healthy subjects and patients had no contraindications to TMS (Rossi et al., 2009) and no history of psychiatric or neurological disease. Right-handedness was confirmed by the Edinburgh handedness inventory (Oldfield, 1971).

The purpose of both experiments was to modulate the ipsilateral CSE in extensor muscles of wrist/fingers, e.g., the ipsilateral extensor digitorum communis (iEDC).

For healthy subjects, we designed a protocol based on existing literature and previous findings of our group to enhance ipsilateral CSE (Figure 1A). The experiment consisted of three conditions (DIS_{Ext}, DIS_{Flex}, and DIS): A DIS protocol (Cash et al., 2016, details see below) was paired with contralateral finger and wrist extension. Four stimulation doublets (i.e.,, eight stimuli) were applied at the time of movement (during the extension phase) in four runs each fifteen trials. Altogether, 480 stimuli were applied during 60 trials. To test for associativity of the intervention, the same DIS protocol was applied during flexion task, i.e., contraction of antagonist muscle (wrist and finger flexion, DIS_{Flex}), or a mental calculating task (iterated multiplications by 2) (Kanthack et al., 2017), i.e.,, *without* a motor task (DIS condition).

For the patients, we designed a protocol based on results from the healthy subject group to enhance ipsilateral CSE, i.e., significant facilitation of ipsilateral CSE via DIS_{Ext} (Figure 1B). The experiment consisted of two conditions (DIS_{Ext}, DIS_{sham}): The same DIS protocol as for healthy subjects was paired with finger and wrist extension. Four stimulation doublets (i.e.,, eight stimuli) were applied at the time of movement (during the extension phase) in one run containing fifteen trials. Altogether, 120 stimuli were applied during 15 trials. To test for placebo effects, the same protocol was applied with the stimulation coil over the occipital cortex (DIS_{sham} condition) (Khedr et al., 2008).

The material and methods of data acquisition applied here were identical to those of previous studies (Kraus et al., 2016b, 2016a) and are cited accordingly:

Data Acquisition

Electromyography (EMG) 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 (Kraus et al., 2016a). Impedances at all electrodes were kept below 10 kΩ. In a next step, data were transferred for online analysis to MATLAB, where they were stored for offline analysis (Kraus et al., 2016a).

Ag/AgCI AmbuNeuroline 720 wet gel surface electrodes (Ambu GmbH, Germany) were used to record electromyography (EMG) activity from the ipsilateral and contralateral EDC (iEDC and cEDC, respectively), from the ipsilateral FDS (iFDS) and the ipsilateral BB (iBB). Two electrodes were placed on the muscle belly 2 cm apart from each other.

TMS protocol

A biphasic TMS pulse (MagPro-R30 + MagOption MagVenture, Germany) was applied through a figure-of-eight coil (MCF-B70). An orientation of 45 ° to the sagittal plane was chosen so that the induced current was directed posterior to anterior in the first phase, and anterior to posterior for the second phase of the stimulus. Frameless

stereotactic neuro-navigation (TMS Navigator, Localite GmbH, Germany) supported the localization of the TMS target position. Once a template MRI (MNI ICBM152 non-linear symmetric T1 Average Brain) had been registered to the head of each participant, the neuro-navigation system tracked the relative positions of the TMS coil and the participant's head during the experiment whilst keeping the stimulation location constant (Mathew et al., 2016). Participants were seated comfortably in an armchair with their elbows semiflexed; the forearm was pronated, fully relaxed, and supported by the arm of the chair. The representation of the EDC in the left for healthy subjects or contralesional M1 for stroke patients was determined prior to the onset of the experimental session (Kraus and Gharabaghi, 2015, 2016). We used 40 % MSO as initial intensity and the anatomically defined 'hand knob' of M1. Whenever the initial stimulator output did not suffice to elicit muscle evoked potentials (MEPs), we increased the output in steps of 5 %. We ensured that the orientation of the coil remained perpendicular to the central sulcus and 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 (Groppa et al., 2012).

Changes in ipsilateral and contralateral CSE was assessed by applying ten TMS pulses at 80, 90, and 100 % MSO, respectively. Healthy subjects facilitated the ipsilateral CSE with an BB contraction at 30 % MVC (Ziemann et al., 1999).

Disinhibition stimulation (DIS)

Cortical stimulation was performed based on a paired-pulse rTMS protocol, referred to as disinhibition stimulation (DIS) in this manuscript for the sake of simplicity and to remain consistent with previous literature applying the same approach (Cash et al., 2016). DIS consisted of a train of four biphasic TMS doublets (eight pulses) at 110 % RMT. The applied interpulse interval (IPI) of a doublet as well as the interdoublet interval (IDI) to induce SICF and LCD, respectively, were individually adjusted to enhance the impact of DIS on ipsilateral CSE (Sewerin et al., 2011). Specifically, IPIs of 1.3, 1.5, and 2 ms, and IDIs of 200, 250, and 300 ms were investigated for every subject in the beginning of each experiment. If we were unable to detect ipsilateral responses in the patient, we chose an IPI of 1.3 ms and an IDI of 220 ms (Cash et al., 2016).

Motor imagery (MI)

In healthy subjects, each condition consisted of four runs with fifteen trials each. In stroke patients, each condition consisted of one run with six trials each (Cash et al., 2016). Each trial began with a 2 s preparation period, followed by a 6 s GO period with the finger/wrist extension of the right/non-paretic hand, and a 6 s relaxation period. The onset of preparation, GO and relax periods was indicated by the auditory cues 'into the hand', 'go' and 'relax', respectively. Subjects were instructed to perform the motor task during the GO period, and to relax during other periods (Royter and Gharabaghi, 2016; Gharabaghi et al., 2014; Gharabaghi, 2016; Bauer et al., 2015; Vukelić and Gharabaghi, 2015a). DIS was initiated 3 s after the 'go' cue during the GO period, since this was the time when the strongest disinhibition was detected in our previous work (Royter and Gharabaghi, 2016; Gharabaghi et al., 2014; Bauer et al., 2015,2016a; Vukelić and Gharabaghi, 2015a, b; Vukelić et al., 2016).

Clinical assessments for stroke patients

Between the interventions, we measured impairment of the paretic limb using the complete Upper Extremity Fugl-Meyer (FM-UE) scale (Fugl-Meyer et al., 1975). FM-UE tests for 33 movements, rated on an ordinal scale (0–2). The maximum score is 66; lower scores reflecting greater impairment.

Additionally, motor impairment and spasticity of the wrist and fingers were assessed using FM and modified Ashworth spasticity (MAS) (Bohannon and Smith, 1987) scales at multiple time points, i.e., before and after each intervention.

The MAS tests for muscle spasticity, rated on an ordinal scale (0-5). Higher scores reflect greater spasticity.

Data processing

Healthy subjects

For the ipsilateral CSE, data was analyzed from rectified EMG, comparing the post-stimulus EMG activity to the background (BG) EMG activity 100 ms before the stimulus. Ipsilateral muscle evoked potentials (iMEPs; µV ms) were measured using the following equation:

$$\Delta iMEP = \left(iMEP_{amplitude} - BG\right) * iMEP_{duration}$$

where $iMEP_{duration}$ is the period the poststimulus EMG above the BG mean ± 1 standard deviation (SD) (Ziemann et al., 1999). The factor $iMEP_{duration}$ was included in the analysis as healthy subjects had to pre-contract iBB. Probability of iMEPs was calculated as the occurrence of an iMEP above the BG mean ± 1 SD for at least 5 ms on a single trial basis. Latency was the earliest deflection of the EMG that was maintained above the BG mean ± 1 SD for at least 5 ms. For contralateral CSE, the EMG data was inspected during offline analysis, discarding any trials containing muscle preactivation (rectified prestimulus EMG activity above 20 µV). Less than 5 % of all trials were rejected due to contamination by muscle activity. The artifact-free contralateral MEP (cMEP) amplitudes were then measured peak to peak. Latency of cMEP was the earliest deflection of the EMG above the BG mean ± 1 SD. Data were analyzed offline using custom written scripts in MATLAB (R2017b, The MathWorks, Inc., United States).

Stroke patients

For ipsilateral CSE, no muscle preactivation was performed due to the hemiparesis of the stroke patients. Therefore, the iMEP amplitudes were measured peak to peak and baseline corrected ($iMEP_{amplitude}$ – BG). Latency of iMEP was the earliest deflection of the iMEP above the BG mean ± 1 SD. For the contralateral CSE, the EMG data was inspected during offline analysis, discarding any trials containing muscle preactivation (rectified prestimulus EMG activity above 20 µV). Less than 5 % of all trials were rejected due to contamination by muscle activity. The artefact-free cMEP amplitudes were then measured peak to peak. Latency of cMEP was the earliest deflection of the EMG above the BG mean ± 1 SD. Data were analyzed offline using custom written scripts in MATLAB (R2017b, The MathWorks, Inc., United States).

Statistical analysis

Statistical analysis was performed using statistical functions in MATLAB (R2017b, The MathWorks, Inc., United States). Data sets were assessed for equality of variances using Levene's test, a prerequisite for a subsequent analysis of variance (ANOVA), and log-transformed if necessary. To investigate intervention effects on ipsilateral and contralateral CSE, an ANOVA on the dependent variable *MEP* with fixed factors *condition* and *time*, and random effect of *subject* was performed. If significant interactions were found, post hoc -tests were performed using Tukey's test. The alpha level was set at $p \leq 0.05$. Results are expressed as mean ± 95 % confidence interval, unless stated otherwise.

Results

Healthy subjects

For the baseline, the latency of the iMEP (Figure 2; 25.79 ± 0.49) lagged significantly behind the latency of the cMEP (17.94 ± 0.39) ($F_{1,100} = 157.35$; $p < 0.001$).

For the iEDC, ANOVA revealed a significant effect of time and condition interaction on ΔiMEP (Figure 3A; effect of time: $F_{2,5399} = 5.34$, $p = 0.012$; effect of condition: $F_{2,5399} = 0.58$, $p = 0.439$; effect of interaction: $F_{4,5399} = 4.23$, $p = 0.002$). DIS_{EXT} significantly increased ΔiMEP up to 140.4 ± 7.4 % of baseline ($p = 0.004$ and $p = 0.026$ for post 10 and 30, respectively; Tukey's test). No significant increase was observed compared to DIS_{FLEX} ($p = 0.996$ and $p = 0.564$ for post 10 and 30, respectively; Tukey's test) and DIS ($p = 0.996$ and $p = 0.898$ for post 10 and 30, respectively; Tukey's test). At post 30, DIS_{FLEX} significantly increased ΔiMEP to 155.1 ± 7.4 % of baseline ($p < 0.001$; Tukey's test).

For the iFDS, no significant interaction was detected (Figure 3B; effect of time: $F_{2,5399} = 4.84$, $p = 0.018$; effect of condition: $F_{2,5399} = 1.25$, $p = 0.305$; effect of interaction: $F_{4,5399} = 2.27$, $p = 0.059$).

For the cEDC, there was a significant effect of time and condition interaction (Figure 3C; effect of time: $F_{2,5399} = 4.06$, $p = 0.033$; effect of condition: $F_{2,5399} = 0.13$, $p = 0.879$; effect of interaction: $F_{4,5399} = 6.46$, $p < 0.001$). At post 10, DIS_{EXT} significantly increased the MEP to 116.6 ± 1.4 % of baseline ($p < 0.001$; Tukey's test), to 108.1 ± 1.4 % of DIS_{FLEX} ($p = 0.001$; Tukey's test) and 107.3 ± 1.4 % of DIS ($p = 0.004$; Tukey's test). DIS_{FLEX} significantly increased the MEP to 107.6 ± 1.4 % of baseline post 10 ($p = 0.003$; Tukey's test). DIS significantly increased the MEP to 108.8 ± 1.4 % of baseline post 10 ($p = 0.001$; Tukey's test). No significant changes for any of the conditions were observed at post 30.

Stroke patients

For the baseline, the latency of the iMEP (Figure 4; 21.09 ± 0.97) lagged significantly behind the latency of the cMEP (17.37 ± 0.28) ($F_{1,62} = 13.65$; $p < 0.001$).

The mean FM-UE was 6.74 ± 7.4 (Table 1; mean ± standard deviation; range: 0 – 36). We found no change in the FM for wrist (0.24 ± 0.7; mean ± standard deviation; range: 0 – 3) and hand (1.12 ± 2.2; mean ± standard deviation; range: 0 – 10) after each intervention ($F_{1,107} = 0$, $p = 1$). No changes were found in the MAS for wrist (2.69 ± 1.2; mean ± standard deviation; range: 0 – 3) and fingers (2.40 ± 1.1; mean ± standard deviation; range: 0 – 4) before or after the intervention ($F_{1,107} = 0$, $p = 1$).

For the iEDC, ANOVA revealed a significant effect of time and condition interaction on the iMEP (Figure 5A; effect of time: $F_{2,5039} = 0.58$, $p = 0.564$; effect of condition: $F_{1,5039} = 0.58$, $p = 0.454$; effect of interaction: $F_{2,5039} = 7.21$, $p < 0.001$). DIS_{EXT} significantly increased the iMEP up to 127.1 ± 3.7 % of baseline ($p < 0.001$ and $p < 0.001$ for post 10 and 30, respectively; Tukey's test) and up to 128.4 ± 3.7 % of DIS_{SHAM} ($p < 0.001$ and $p = 0.026$ for post 10 and 30, respectively; Tukey's test).

For the iFDS, no significant interaction was detected (Figure 5B; effect of time: $F_{2,5039} = 1.06$, $p = 0.358$; effect of condition: $F_{1,5039} = 0.26$, $p = 0.615$; effect of interaction: $F_{2,5039} = 1.41$, $p = 0.245$).

For the cEDC, there was a significant effect of time, condition, and the interaction (Figure 5C; effect of time: $F_{2,5039} = 0.95$, $p = 0.400$; effect of condition: $F_{1,5039} = 1.90$, $p = 0.180$; effect of interaction: $F_{2,5039} = 11.00$, $p < 0.001$). DIS_{EXT} significantly increased the MEP up to 111.7 ± 1.6 % of baseline ($p = 0.003$ and $p < 0.001$ for post 10 and 30, respectively; Tukey's test) and up to 115.6 ± 1.6 % of DIS_{SHAM} ($p = 0.003$ and $p < 0.001$ for post 10 and 30, respectively; Tukey's test).

Discussion

In this study with healthy subjects and stroke patients, we investigated the optimal protocol to modulate ipsilateral CSE of the EDC, a muscle highly affected in stroke patients. After successful combination of repetitive paired-pulse TMS (DIS) with extension of non-paretic wrist/fingers in healthy subjects, we tested the same plasticity inducing protocol in severely affected stroke patients and were able to induce lasting changes for the ipsilateral paretic muscle.

Ipsilateral pathways

In both healthy subjects and stroke patients, the latency of the iMEP measured in the iEDC (25.79 ± 0.49 and 21.09 ± 0.97, respectively) differed significantly from the latency of the cMEP in the cEDC (17.94 ± 0.39 and 17.37 ± 0.28, respectively). Differences in healthy subjects may be slight underestimates of iMEP latency, as preactivation of ipsilateral muscle slightly shortened latencies by approximately 1 ms (Alagona et al., 2001). Nevertheless, the observed latency difference is similar to the lag described in literature (Ziemann et al., 1999; Bradnam et al., 2010; Tazoe and Perez, 2014; McCambridge et al., 2016) and points towards the involvement of an oligosynaptic pathway or a longer conduction distance from excited cortical areas to the origin of the descending pathway. These descending pathways may originate in the medial brainstem and include the reticulospinal pathway (Riddle et al., 2009; Baker and Perez, 2017) and the rubrospinal pathway (Ishida et al., 2016). Another possibility are midline crossing fibers of the contralateral CST (Starkey et al., 2012; Wahl et al., 2017). It is unlikely that ipsilateral responses were affected by the opposite hemisphere, as transcallosal activation requires approximately 10-20 ms (Cracco et al., 1989; Meyer et al., 1998) and is therefore too long to account for the delays we found between the onset of cMEP and iMEP. However, detailed studies investigating the pathways of the iCST in healthy subjects and stroke patients are required.

Disinhibition protocols

In this study, we analyzed the combination of two disinhibition protocols, each targeting GABAergic interneuron networks, as a decrease of GABAergic inhibition has been linked to motor learning and recovery (Lazar et al., 2010; Blicher et al., 2015). Paired

pulse TMS enables targeting of intracortical circuits (Kujirai et al., 1993; Ziemann et al., 1996). An initial conditioning stimulus evokes multiple descending volleys (I-waves) at a periodicity of about 1.5 ms from transynaptic activation of principal cells through excitatory interneuronal connections (Di Lazzaro et al., 2012). These I-waves cause time-dependent modulation of the MEP response to subsequent stimuli and allows the targeting of $GABA_B$ergic interneuron networks, resulting in a disinhibition of CSE (Cash et al., 2010, 2016). Another method to disinhibit CSE is the active movement of a limb (Goodwill et al., 2012; Chiou et al., 2013), e.g., active extension of the contralateral or non-paretic wrist/fingers for healthy subjects or stroke patients, respectively. Unilateral motor execution has been shown to modulate bilateral CSE (Hortobágyi et al., 2003; Zijdewind et al., 2006; Stippich et al., 2007; Perez and Cohen, 2008; Chiou et al., 2013) and to disinhibit ICI (Goodwill et al., 2012; Chiou et al., 2013). Taken together, targeting $GABA_B$ receptors via DIS (Cash et al., 2010, 2016) in combination with targeting both $GABA_A$ and $GABA_B$ receptors via voluntary motor execution (Goodwill et al., 2012; Chiou et al., 2013) is a potential mechanism to release the contralesional M1 from GABAergic mediated inhibition.

Enhancement of ipsilateral connections

In both healthy subjects and stroke patients, DIS stimulation in combination with extension of the wrist and fingers resulted in a significant increase of the iCSE up to 140.4 ± 7.4 % and 127.1 ± 3.7 % compared to baseline measurements, respectively. Motor execution leads to a bilateral event-related desynchronization (ERD) (Hasegawa et al., 2017) and might thereby modulate the plasticity inducing effect of DIS.

The decreased effect of DIS_{EXT} in stroke patients compared to healthy subjects might be attributed to different duration of intervention, e.g., 15 minutes for healthy subjects and 1.5 minutes for stroke patients. As 1.5 minutes has been shown to be effective for plasticity induction in the contralateral CST (Cash et al., 2016), we chose the shorter intervention duration for stroke patients in order to decrease costs and time required by both the patient and the TMS specialist in regard to future therapeutic interventions. Additionally, increased stimulus dosage appears to also increase the potential risk of adverse events, including seizure and headache (Rossi et al., 2009). The difference in study designs limits comparability between the two groups. Furthermore, changes due to the intervention duration in stroke patients needs to be addressed in future studies. Nevertheless, DIS_{EXT} for 1.5 min resulted in a significant increase of ipsilateral CSE. The observed plasticity induction might even be further enhanced with a longer intervention duration.

Limitations and future perspectives

Multi-dimensional models suggest the consideration of structural damage and availability of residual motor pathways (Bradnam et al., 2013; Di Pino et al., 2014; Plow et al., 2016), i.e., rehabilitation of less affected patients should base on ipsilesional M1, while more affected patients rely on contralesional M1 for recovery. These models underline the urgency for biomarkers that allow therapies to be selected and tailored to patient subgroups. DIS_{EXT} is a potential therapy that requires further investigation in a larger stroke patient cohort in order to determine the patient subgroup which profits most from this intervention.

We found only significant changes in the ipsilateral CSE but did not observe any differences in motor behaviour of patients. This might be in correlation with the duration

of intervention. A therapeutic intervention over a longer duration, even days, may result in positive changes not only of iCSE but also of motor output of the patient. This is supported by the observation that clinical effects are often short-lived and multiple intervention sessions seem to extend clinical benefit (Bäumer et al., 2003; Edwards et al., 2008). The required duration as well as the frequency of application need to be further addressed in future studies.

Furthermore, DIS_{EXT} has the potential to adjust the brain into a more receptive state, e.g., plastic, and the clinical outcome may therefore be further improved upon a combination with physiotherapy (Talelli and Rothwell, 2006; Edwards et al., 2008).

Another limitation was that for stroke patients we decided on a single-blinded design, but blinding investigators as well would be beneficial in future studies to avoid bias of the experimenter. However, due to the shorter stimulation duration we decided against a double-blinded study, as it would have been too time consuming to exchange the experimenter during the intervention like we did in the healthy subjects.

If optimal interval for paired-pulse TMS was not detectable during the session in patients, we chose intervals based on existing literature (Thickbroom et al., 2006; Cash et al., 2016). However, it was shown that optimizing interval to individual optimum is favorable (Sewerin et al., 2011). Future studies should therefore include first a screening session followed by the interventional session to ensure the optimization of intervals for each patient.

Conclusion

In conclusion, DIS_{EXT} was able to modulate ipsilateral CSE in both healthy subjects and stroke patients and is therefore a powerful tool for plasticity induction in a neurorehabilitative framework. These findings provide a therapeutic backdoor to modulate ipsilateral pathways. Furthermore, they highlight the importance of ipsilateral projecting routes from the contralesional M1 when the ipsilesional connections are no longer a viable target for plasticity enhancing interventions.

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Tables

Table 2. Patient characteristics. Age (years, y), Hem = hemisphere affected by stroke, Hand = hand dominance before stroke, time = time since stroke (months, m), Fugl-Meyer upper extremity (FM-UE) score (maximum 66), wrist score (maximum 10), hand score (maximum 14), modified Ashworth scale for finger spasticity (MAS-F; maximum 5), modified Ashworth scale for wrist spasticity (MAS-W; maximum 5).

PAT	AGE (Y)	SEX	HAND	HEM	TIME (M)	FM-UE	WRIST	HAND	MAS-F	MAS-W
1	45	M	R	R	32	2	0	0	3	2
2	74	M	R	L	93	2	0	0	2	3
3	57	M	R	L	68	0	0	0	3	3
4	74	F	R	R	27	10	0	0	3	3
5	65	M	R	L	8	4	0	0	3	4
6	58	M	R	R	58	4	0	1	3	3
7	59	M	R	R	150	4	0	0	3	4
8	66	M	R	R	237	3	0	0	3	4
9	49	F	R	R	79	24	2	4	2	2
10	77	M	R	R	203	4	0	0	3	3
11	66	F	R	L	19	9	0	5	3	4
12	64	F	R	R	10	6	0	0	4	4
13	63	M	R	R	99	2	0	0	4	4
14	59	M	R	L	142	4	0	1	3	4
15	74	F	R	R	18	3	0	0	3	3
16	44	F	L	R	19	13	3	5	1	1
17	74	F	R	L	55	3	0	0	3	3
18	51	F	R	L	211	5	0	0	3	4
19	57	F	R	R	41	11	1	0	4	4
20	51	F	R	L	10	6	0	0	2	2
21	51	F	R	L	89	4	0	0	3	2
22	74	F	R	L	15	2	0	0	1	1
23	67	M	R	L	110	3	0	0	1	1
24	62	M	R	R	43	6	0	0	3	4
25	67	M	R	L	30	6	0	2	0	1
26	54	M	R	R	57	29	0	10	0	1
27	54	M	R	R	122	6	0	1	0	0
MEAN	61				76	7	0	1	2	3
SD	9				64	7	1	2	1	1

Figures

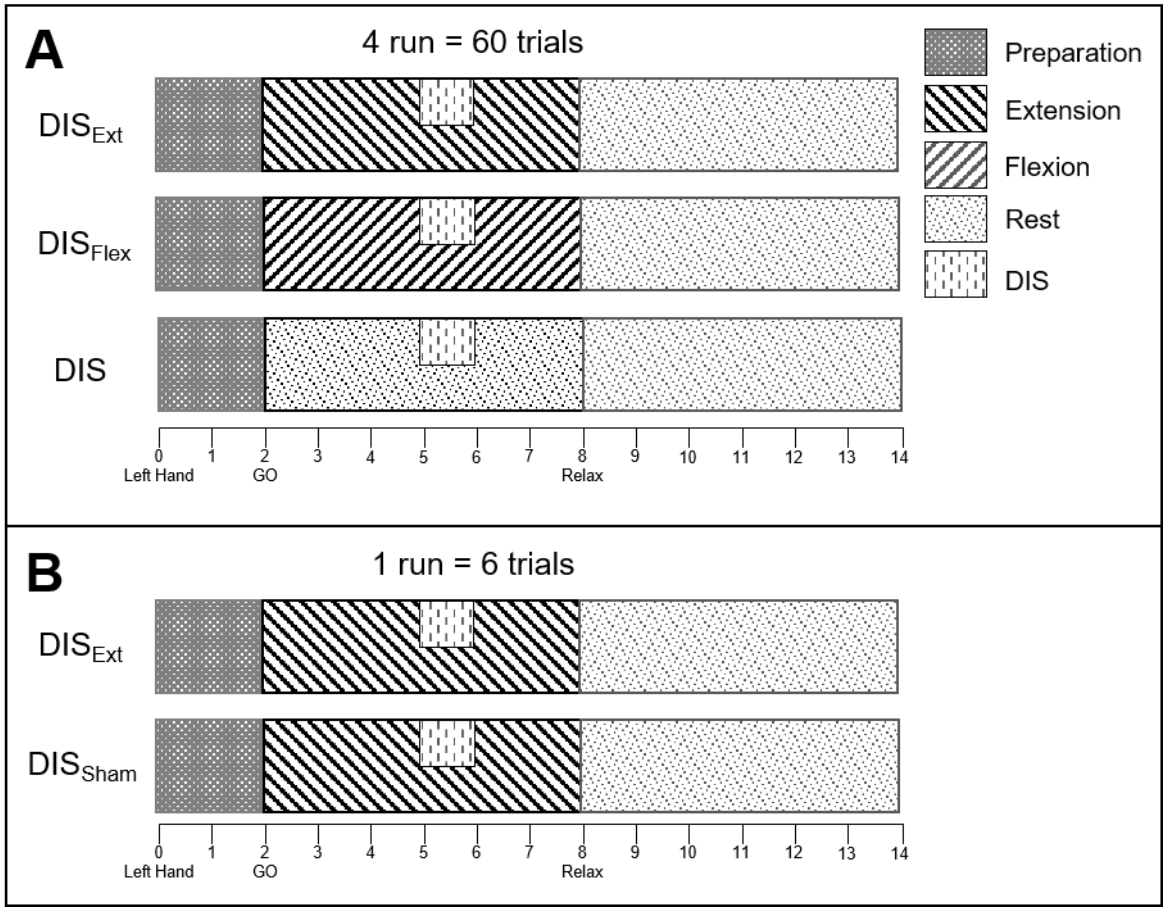

Figure 7. Study design showing the building blocks of each experiment.

A: For the healthy subjects, the experiment consisted of three conditions with four runs á 15 trials (total of 60 trials). DIS was paired with finger/wrist extension (DIS_{EXT}). As controls, DIS was applied during finger/wrist flexion (DIS_{FLEX}) or during rest (DIS).

B: For stroke patients, the experiment consisted of two conditions with one run containing six trials. DIS was paired with finger/wrist extension (DIS_{EXT}). As a control, DIS was applied with the coil over the parietal cortex (DIS_{SHAM}) instead of the motor cortex.

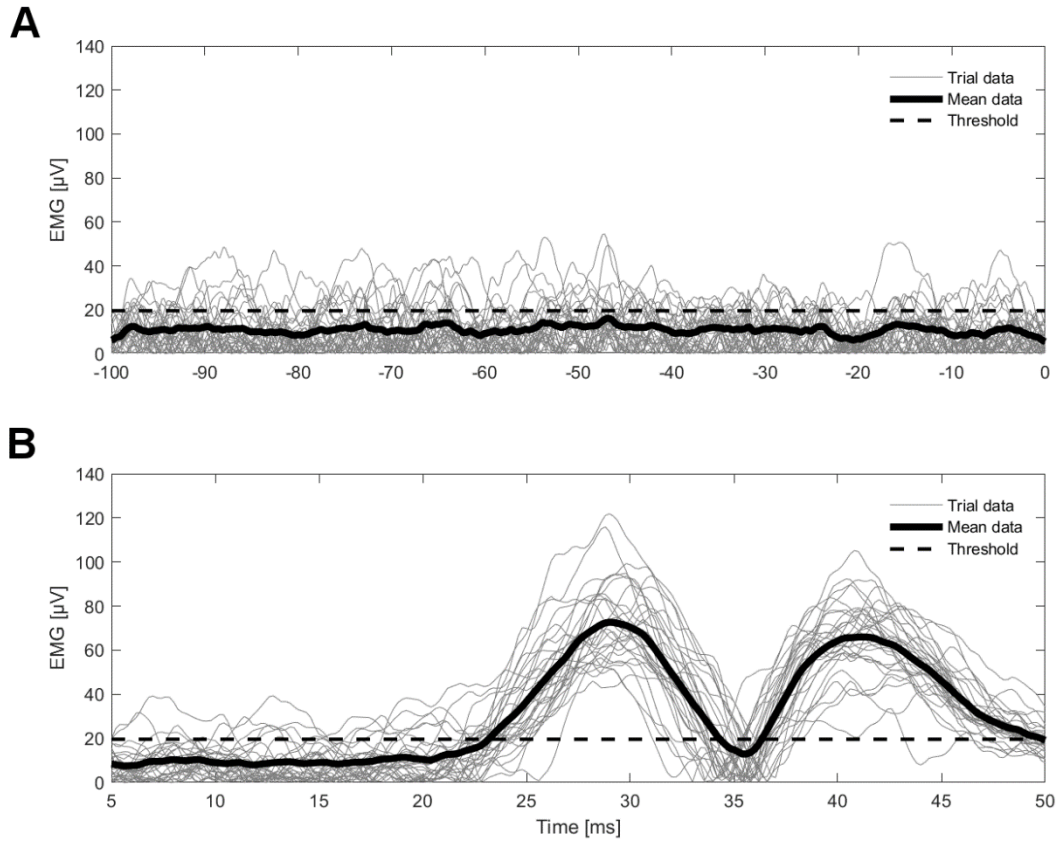

Figure 2: Rectified electromyography (EMG) recordings from the ipsilateral EDC (iEDC) of a representative participant. The averaged trace is shown in black, single trial data is shown in gray. The threshold for detection was the mean EMG of background (BG) + 1 standard deviation (SD) of BG

A: BG activity of the iEDC during contraction of the BB.

B: EMG from iEDC after TMS. Ipsilateral MEPs were measured as ($iMEP_{amplitude}$ - BG)*$iMEP_{duration}$.

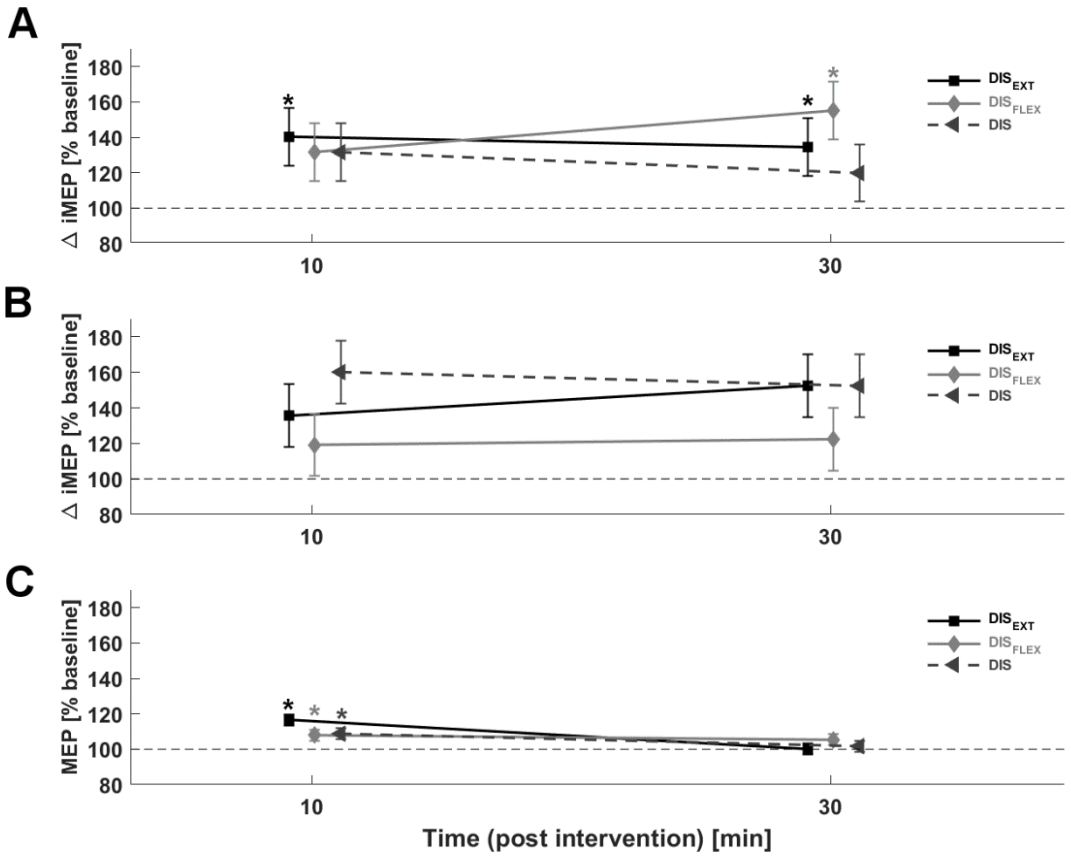

Figure 3. Time-course of ΔiMEP changes post intervention for iEDC (A) and iFDS(B), and of MEP changes post intervention for cEDC (C).

A: DIS_{EXT} showed a significant increase of ipsilateral CSE for the iEDC 10 and 30 min after the intervention. DIS_{FLEX} showed a significant increase 30 min after the intervention (* indicates $p < 0.05$, Tukey's test).

B: No changes were observed for iFDS.

C: DIS_{EXT} showed the strongest however not lasting increase of MEPs for the cEDC after the intervention (* indicates $p < 0.05$, Tukey's test).

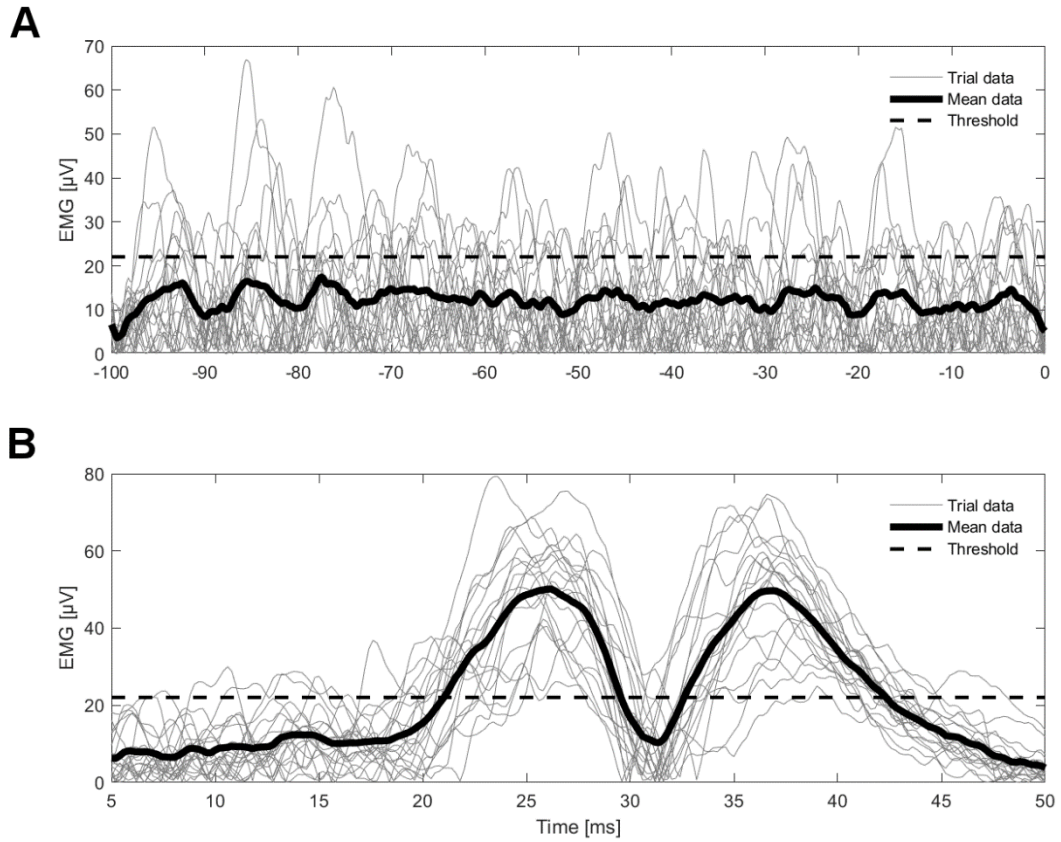

Figure 4: Rectified electromyography (EMG) recordings from the paretic ipsilateral EDC (iEDC) of a representative patient during rest. The averaged trace is shown in black, single trial data is shown in gray. The threshold for visual inspection was the mean EMG of background BG + 1 standard deviation (SD) of BG

A: (BG) activity of the iEDC.

B: EMG from iEDC after TMS. Ipsilateral MEPs were measured as peak to peak.

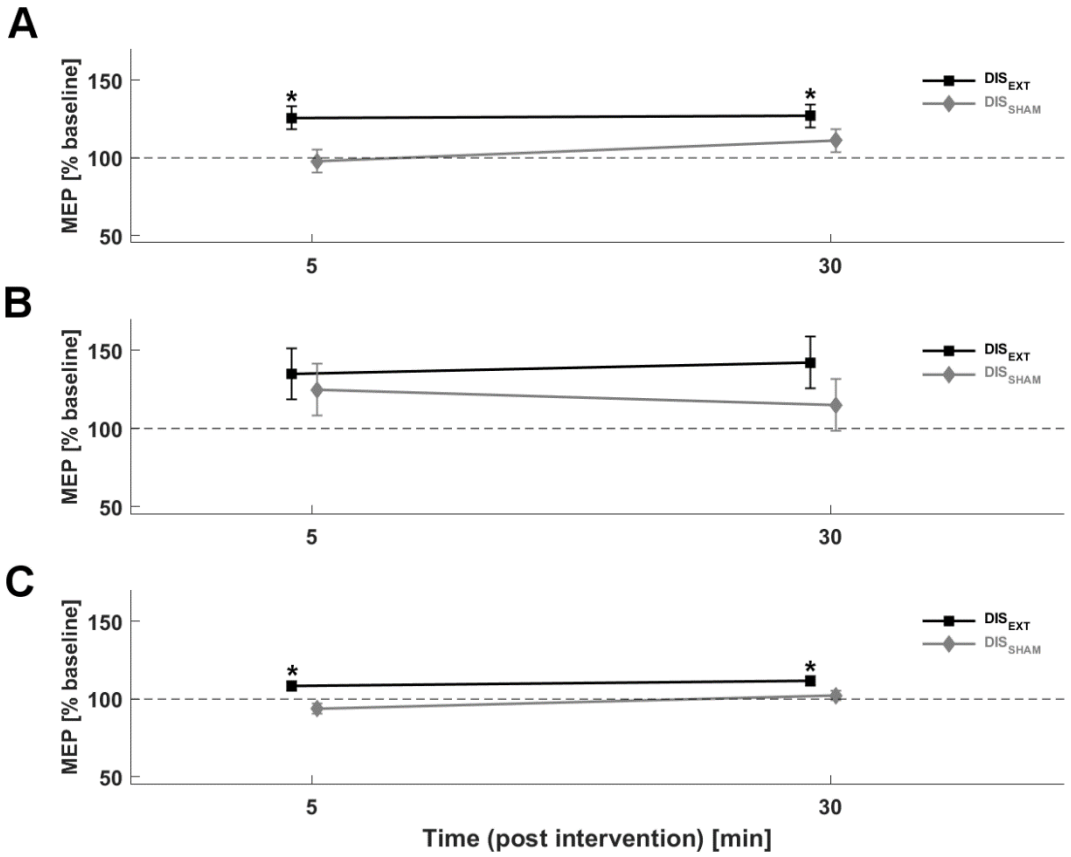

Figure 5. Time-course of MEP changes post intervention for iEDC (A), iFDS(B), and cEDC (C).

A: DIS_{EXT} showed a significant and persistent increase of ipsilateral CSE for the iEDC after the intervention (* indicates $p < 0.05$, Tukey's test).

B: No changes were observed for the iFDS.

C: DIS_{EXT} showed a significant and consistent increase of MEPs for the cEDC after the intervention (* indicates $p < 0.05$, Tukey's test).