KU LEUVEN

GROEP BIOMEDISCHE WETENSCHAPPEN

FACULTEIT BEWEGINGS- EN REVALIDATIEWETENSCHAPPEN

Unravelling ipsilateral interactions between the left dorsal premotor and primary motor cortex

door Ruben Debeuf
en Sybren Van Hoornweder

masterproef aangeboden tot het behalen van de graad van Master of Science in de revalidatie-wetenschappen en kinesitherapie

o.l.v.
Dr. K. Cuypers, promotor

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VOORWOORD

Beste lezer, via dit voorwoord willen we graag iedereen bedanken die essentieel was voor de uitwerking van deze masterproef.

Vooreerst willen we onze promotor doctor Koen Cuypers uitdrukkelijk en welgemeend bedanken. Zonder zijn begeleiding, kennis en het vlotte onderlinge contact was het maken van deze masterproef onmogelijk geweest. Ondanks enkele technische en structurele tegenslagen konden we steeds rekenen op een aangename, motiverende en productieve wisselwerking met doctor Cuypers. Doordat we bij het volledige onderzoeksproces zeer nauw betrokken waren en veel inspraak kregen, was dit een uitermate verrijkende en leerrijke ervaring die smaakt naar meer.

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Ten slotte willen we onze vrienden en familie bedanken. Dit voor de directe en de indirecte steun en alle kansen die zij steeds aan ons gegeven hebben.

We wensen u veel leesplezier toe.

Zwevegem, 15 april 2020 R.D.
Maldegem, 15 april 2020 S.V.H.

SITUERING

Deze masterproef werd uitgevoerd binnen de eenheid 'Movement Control & Neuroplasticity' (KU Leuven). Deze onderzoeksgroep staat onder leiding van Professor Swinnen en richt zich op verschillende facetten binnen het neurowetenschappelijk onderzoek. Zo wordt onder andere de functie van bepaalde hersenregio's met betrekking tot handbewegingen bestudeerd. Het is binnen dit thema dat deze scriptie te kaderen valt. Meer specifiek spitst deze thesis zich toe op de identificatie van een protocol dat de functionele relatie van twee nabijgelegen hersenregio's kan onderzoeken. De hersenregio's in kwestie zijn de linker dorsale premotorische cortex (PMd) en linker primair motorische handregio ($M1_{hand}$).

Over $M1_{hand}$ is geweten dat deze hersenregio de motorische representatie van alle handspieren bevat en zo deze spieren kan aansturen (He et al., 1993). Over de andere hersenregio (PMd) en voornamelijk de functionele invloed die PMd op $M1_{hand}$ uitoefent, is minder geweten. Voorgaande studies hebben reeds aangetoond dat beide hersenregio's directe verbindingen met elkaar hebben (Dum & Strick, 1991; Dell'Acqua et al., 2011). De functionele rol van deze verbindingen is echter nog ongekend maar kan onderzocht worden door middel van transcraniële magnetische stimulatie (TMS). Hierbij wordt via een magnetisch veld, een elektrische stroom opgewekt in de cortex (Chris & Reza, 2006). Wanneer een TMS-puls over $M1_{hand}$ wordt gegeven, resulteert dit in een contractie van een handspier. De exacte locatie waar de puls wordt toegediend, bepaalt welke handspier contraheert.

TMS wordt frequent gebruikt om de verbindingen tussen PMd en $M1_{hand}$ te onderzoeken. De focus lag in het verleden voornamelijk op linker PMd - rechter $M1_{hand}$ connectiviteit omwille van technische limitaties. De enkele studies die de invloed van linker PMd op linker $M1_{hand}$ hebben onderzocht, gebruikten een TMS-setup die het niet toelaat gedurende lange tijdsperiodes te stimuleren (Civardi et al., 2001; Groppa et al., 2012a; Groppa et al., 2012b). In de huidige studie zal een TMS-configuratie gebruikt worden die dit wel toelaat. Vier protocollen, gebaseerd op voorgaande studies (Civardi et al., 2001, Groppa et al., 2012a), zullen uitvoerig worden getest. Indien een protocol wordt geïdentificeerd waarmee op betrouwbare wijze de interactie tussen PMd en $M1_{hand}$ kan worden onderzocht, zal dit in de toekomst gebruikt worden voor verder onderzoek tijdens meer functionele handtaken.

Deze masterproef is een technisch en fundamenteel onderzoek, maar draagt als basis voor verder onderzoek een zekere maatschappelijke relevantie. Indien significante resultaten worden gevonden, kan men deze gebruiken om het werkingsmechanisme van $M1_{hand}$ en PMd beter te doorgronden. Een toegenomen inzicht in het proces achterliggend aan handbewegingen kan een startpunt zijn voor nieuwe therapieën en behandelingsmodaliteiten op lange termijn.

Referenties

Chris H, Reza J (2006) The guide to magnetic stimulation. In, p 45: Magstim.

Civardi C, Cantello R, Asselman P, Rothwell JC (2001) Transcranial magnetic stimulation can be used to test connections to primary motor areas from frontal and medial cortex in humans. Neuroimage 14:1444-1453.

Dell'Acqua F, Vergani F, Malik F, Hodge H, Roy P, Valabregue R, Thiebaut de Schotten M (2011) Short frontal lobe connections of the human brain. Cortex; a journal devoted to the study of the nervous system and behavior 48:273-291.

Dum RP, Strick PL (1991) The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci 11:667-689.

Groppa S, Schlaak BH, Munchau A, Werner-Petroll N, Dunnweber J, Baumer T, van Nuenen BF, Siebner HR (2012a) The human dorsal premotor cortex facilitates the excitability of ipsilateral primary motor cortex via a short latency cortico-cortical route. Hum Brain Mapp 33:419-430.

Groppa S, Werner-Petroll N, Munchau A, Deuschl G, Ruschworth MF, Siebner HR (2012b) A novel dual-site transcranial magnetic stimulation paradigm to probe fast facilitatory inputs from ipsilateral dorsal premotor cortex to primary motor cortex. Neuroimage 62:500-509.

He SQ, Dum RP, Strick PL (1993) Topographic organization of corticospinal projections from the frontal lobe: motor areas on the lateral surface of the hemisphere. J Neurosci 13:952-980.

ABSTRACT

Few studies have identified the intrahemispheric functional connectivity between the left dorsal premotor cortex (PMd) and the left primary motor hand area ($M1_{hand}$). This proof of concept study set out to evaluate four intrahemispheric dual-site transcranial magnetic stimulation (dsTMS) paradigms probing PMd - $M1_{hand}$ connectivity, based on two previous studies (Civardi et al., 2001; Groppa et al., 2012a). A novel dsTMS setup combining a cooled and a non-cooled coil was used which ensures long periods of testing. Nine right-handed subjects of both sexes participated in the main experiment. Analysis of the data revealed that stimulating PMd 6 ms prior to $M1_{hand}$ increased the size of peak to peak motor evoked potentials (MEPs) measured in the right first dorsal interosseus muscle by 20% on average. PMd was stimulated at an intensity of 75% resting motor threshold with the second TMS pulse wave inducing a latero-medial current in the cortex. $M1_{hand}$ was stimulated at an intensity that initially evoked MEPs with an amplitude of 1 mV with the second pulse wave inducing a postero-anterior current in the cortex. Further research can use these findings as a starting point.

Key words: transcranial magnetic stimulation; dorsal premotor cortex; primary motor cortex

SIGNIFICANCE STATEMENT

Controlled use of the hands is fundamental to humans. Two regions associated with this are the primary motor hand area ($M1_{hand}$) and dorsal premotor cortex (PMd). We extensively tested four intrahemispheric dual-site transcranial magnetic stimulation (dsTMS) paradigms probing the interaction between left PMd and left $M1_{hand}$. A novel dsTMS setup combining a cooled and a non-cooled coil was used for testing. Our results identified one protocol able to probe PMd - $M1_{hand}$ connectivity in a reliable way. More precisely, when using this dsTMS paradigm, PMd yielded a facilitatory influence over $M1_{hand}$, increasing motor evoked potentials measured at the right first dorsal interosseus muscle with approximately 20%. These results can be a cornerstone for future research.

INTRODUCTION

Being able to use your hands in a controlled manner is of extreme importance as manipulation of objects is an intrinsic part of everyday life. The extensive control exhibited by the primary motor hand area ($M1_{hand}$) over the hand muscles through monosynaptic projections to the cervical motor neurons is well documented in both humans and primates (He et al., 1993; Hlustik et al., 2001; Lemon, 2008). Full mapping of the primary motor area in humans was first done by Boldrey and Penfield (1937), displaying the cortical representations of the hand muscles. Of course, $M1_{hand}$ is not the only region related to the preparation and execution of hand movements. Among others, the supplementary motor area and ventral and dorsal premotor areas are involved through connections to $M1_{hand}$ (Dell'Acqua et al., 2011; Toga, 2015: Genon et al., 2018) but also through direct connections to the spinal cord (Dum and Strick, 1991; Toga, 2015;). The current study focusses on the intrahemispheric temporal influence of the left dorsal premotor cortex (PMd) on left $M1_{hand}$.

PMd is a lateralized and heterogeneous structure with various subregions that each possess different functional properties (Genon et al., 2016; Genon et al., 2018). Subdivisions can be made based on the presence of a rostro-caudal gradient, which states that the more rostral areas are involved in cognitive and pre-movement processes while the caudal areas primarily play a role in movement control (Picard and Strick, 2001; Chouinard and Paus, 2006; Abe and Hanakawa, 2009; Genon et al., 2018). A recent study divided left PMd in five subregions based on a rostro-caudal and ventro-dorsal axis (Fig. 1) (Genon et al., 2018). One of the distinguished subregions is the caudal subregion, associated with action execution and motor learning (Genon et al., 2018). This finding is in line with previous studies indicating that left PMd plays a dominant role in left and right hand movements, exerting influence on both left and right $M1_{hand}$ (Rizzo et al., 2004; Baumer et al., 2006; O'Shea et al., 2007; Castiello and Begliomini, 2008; Duque et al., 2012; Fujiyama et al., 2016). Furthermore, the existence of anatomical connections between ipsilateral PMd and $M1_{hand}$ has already been confirmed (Guye et al., 2003; Chouinard and Paus, 2006; Raos et al., 2006; Dell'Acqua et al., 2011). The functional and temporal properties of PMd - $M1_{hand}$ connectivity have also been investigated with the majority of studies focusing on the contralateral influence of left PMd on right $M1_{hand}$ (Mochizuki et al., 2004; O'Shea et al., 2007; Bestmann et al., 2008; Moisa et al., 2012; Fujiyama et al., 2016; Fiori et al., 2017).

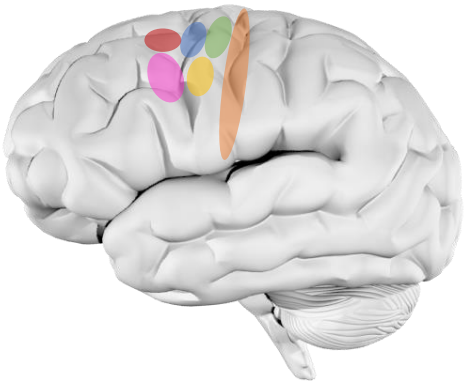


Figure 1. Left brain hemisphere with the primary motor cortex (orange) and the five subregions of the dorsal premotor cortex (rostral: red, rostro-ventral: pink, central: blue, caudal: green, ventral: yellow). Adapted from Genon et al. (2018)

Research identifying ipsilateral temporal connectivity is rather scarce (Civardi et al., 2001; Koch et al., 2007; Groppa et al., 2012a; Groppa et al., 2012b; Ni et al., 2015). Probing ipsilateral interactions is indeed challenging due to the combination of the close proximity of the stimulation targets and the size of the stimulation coils. Imaging studies revealed that the caudal part of PMd lies approximately 9 mm anterior of $M1_{hand}$ (Picard and Strick, 2001). Coordinates for PMd stimulation in dual-site transcranial magnetic stimulation (dsTMS) studies vary widely but range between 20 and 60 mm anterior to $M1_{hand}$ (mean= 32 mm) (Civardi et al., 2001; Groppa et al., 2012a; Ni et al., 2015; Fiori et al., 2017; Fricke et al., 2019).

Transcranial magnetic stimulation (TMS) is a commonly used method to identify cortical excitability in a safe, non-invasive way (Wassermann, 1998; Andrew, 2007; Rossi et al., 2009). Through a magnetic field, electrical currents are induced in the cortical surface (Rossini et al., 2015). Typically, TMS devices produce a monophasic or a biphasic pulse. A monophasic pulse consists of a monodirectional current dampened after the first quarter cycle (Delvendahl et al., 2014), whereas a biphasic pulse is composed of two half segments with opposite current direction (Delvendahl et al., 2014). The application of a sufficiently strong TMS pulse over $M1_{hand}$ leads to the generation of direct (D-waves) and indirect waves (I-waves) (Patton and Amassian, 1954; Day et al., 1989). D-waves represent the direct excitation of the corticospinal axon (Terao and Ugawa, 2002), while I-waves seem to be the result of presynaptic activation although their exact origin is not yet fully comprehended (Di Lazzaro et al., 2012; Niemann et al., 2018). When administering a biphasic TMS pulse with the second pulse wave inducing a postero-anterior (PA) current over $M1_{hand}$, I-waves are predominantly generated (Di Lazzaro et al., 1998). The resulting effect can be measured in the targeted muscle as a motor evoked potential (MEP) (Chris and Reza, 2006; Andrew, 2007; Rossi et al., 2009).

To probe the functional and temporal interaction between two brain regions, dsTMS is frequently employed. This paradigm, in which two coils are placed over two distinct brain regions, was first described by Ferbert et al. (1992) and has already been applied in a multitude of studies involving inter- and intrahemispheric PMd - $M1_{hand}$ connectivity (Civardi et al., 2001; Mochizuki et al., 2004; Koch et al., 2007; O'Shea et al., 2007; Bestmann et al., 2008; Groppa et al., 2012a; Groppa et al., 2012b; Moisa et al., 2012; Fujiyama et al., 2016). Besides dsTMS, another modality often used to identify the functional connectivity between brain regions in general and left PMd and $M1_{hand}$ in particular, is functional magnetic resonance imaging (fMRI) (Picard and Strick, 2001; Guye et al., 2003; Beets et al., 2015). Although fMRI has excellent spatial resolution, the technique faces some shortcomings such as low temporal resolution and the inability to identify the precise nature of connections (Glover, 2011).

fMRI cannot differentiate between relevant and irrelevant brain activity for a certain (motor) behavior (Ramsey et al., 2010). TMS on the other hand, can circumvent this drawback. As TMS directly manipulates neural activity, it can evince a causal relationship between a cortical region and a certain (motor) behavior. Furthermore, TMS has excellent temporal resolution and relatively good spatial specificity (Wagner et al., 2007; Ruff et al., 2009; Cazzato, 2010). Although spatial properties depend on the geometrics of the used coil, resolution can be in the order of millimeters (Bolognini and Ro, 2010; Sliwinska et al., 2014).

Two authors previously investigated the effect of stimulation over PMd and $M1_{hand}$ versus $M1_{hand}$ alone using single pulse dsTMS (Civardi et al., 2001; Groppa et al., 2012a) and demonstrated the existence of a direct state-dependent premotor-to-motor pathway (Groppa et al., 2012a). Using dsTMS, Civardi et al. (2001) found that a conditioning stimulus (CS) applied 60 mm anterior of left $M1_{hand}$ over left PMd prior to a test stimulus (TS) over left $M1_{hand}$, elicited changes in MEPs. An interstimulus interval (ISI) of 6 ms proved to be most effective in suppressing the MEPs of the right first dorsal interosseus muscle (FDI). Notably, it was reported that changing the current direction over the premotor areas resulted in different MEP-amplitudes. An antero-posterior (AP) current induced a decrease in MEP-amplitude, while a PA current yielded no effect (Civardi et al., 2001). Groppa et al. (2012a) applied dsTMS to $M1_{hand}$ and PMd in the inversed temporal order. First left $M1_{hand}$ received a TS, followed by a CS targeting left PMd. The most effective ISI for MEP facilitation was 2.8 ms (Groppa et al., 2012a). The rationale behind this temporal inversion is the following: when a TMS pulse is given over $M1_{hand}$, the late and final I-waves leave the cortex several milliseconds after the pulse is administered (Day et al., 1989; Di Lazzaro et al., 1998). With PMd and $M1_{hand}$ being directly connected (Dum and Strick, 2005; Castiello and Begliomini, 2008), it can be assumed that premotor-to-motor conduction should take 2 ms at most (Groppa et al., 2012a). A TMS pulse applied to PMd shortly after a pulse over $M1_{hand}$ should thus still be able to facilitate late I-wave generation. Groppa et al. (2012a) used figure-of-eight coils with a drop-like geometry, the authors claim that this enabled them to minimize the distance between the areas stimulated to approximately 20 mm.

Contrary to the paradigms used by Civardi et al. (2001) and Groppa et al. (2012a), here a novel intrahemispheric dsTMS configuration was evaluated. This configuration consisted of a small cooled and a large non-cooled figure-of-eight coil and ensured long periods of stimulation. Using a cooled coil is important since the ambition of future research is to investigate ipsilateral interactions during movement preparation of a motor coordination task, requiring several stimulation conditions and multiple repetitions per condition.

We hypothesize that the influence left PMd exerts on left $M1_{hand}$ is state dependent and thus strongly reliant on parameters such as current intensity, direction and timing. Regarding what current flow is most proficient in the coil stimulating left PMd, stating a hypothesis is difficult. Reversing the current direction in the coils targeting the premotor areas, among other brain areas, has shown to significantly change the induced effect of TMS (Civardi et al., 2001; Kammer et al., 2001; Balslev et al., 2007; Janssen et al., 2015; Hannah and Rothwell, 2017; Vink et al., 2018). A small pilot experiment was conducted prior to the main study to further confirm this hypothesis.

MATERIALS AND METHODS

Participants

In the pilot, two healthy adult men [aged 29.5 ± 8.5 years (mean ± standard deviation (SD))] participated. Thirteen healthy subjects were screened for the main experiment, nine participants were found to be eligible [aged 22.4 ± 1.3 years; 6 males]. Four participants were excluded as the maximum stimulation intensity of the used coils could not reliably evoke MEPs in these subjects. All subjects were consistently right-handed according to the Edinburgh handedness inventory [mean score: 94.4% SD = 8.3, >0.75] (Oldfield, 1971). The study was approved by the local Medical Ethics Committee of KU Leuven (study number: S60428) in accordance to the Declaration of Helsinki and its amendments (World Medical Association 1964, 2008). Each subject read and signed a written informed consent along with a safety questionnaire prior to the experiment and received financial compensation for participating.

EMG Recordings

EMG signals were recorded using surface Ag-electrodes (Bagnoli™ DE-2.1 EMG Sensors, DELSYS Inc, Boston, MA, USA) placed over the right FDI belly with single-use double-sided adhesive skin interfaces (DELSYS Inc, Boston, MA, USA). The reference electrode was placed on the bony parts of the dorsal wrist. Raw signals were amplified and bandpass filtered (20 Hz – 2000 Hz). Filtering was done for 50/60 Hz noise through a Humbug Noise Eliminator (Digitimer, Hertfordshire, United Kingdom). Digitization of the signals was done using Signal (version 6.05, Cambridge Electronic Design, UK).

Pilot experiment

Initially, the location of $M1_{hand}$, more precisely the motor representation of the right FDI, was determined through hotspotting with an MC-B35 coil (MagVenture A/S, Farum, Denmark) (outer diameter: 47mm). The coil was connected to a MagPro X100 stimulator (MagVenture A/S, Farum, Denmark) with MagOption module, enabling the change of current direction. The coil handle was positioned tangentially to the scalp with the short axis being approximately parallel with the central sulcus (Fig. 2a) (Groppa et al., 2012a). Biphasic pulses were administered with the second pulse inducing a PA current in the cortex. The hotspot that elicited a maximal MEP in the FDI with minimal stimulation strength was located in a systematic fashion. First, stimulus intensity was chosen to be suprathreshold in line with Groppa et al. (2012a). Next, the coil was moved to coordinates allocated on a grid projected on a population representative MRI scan [Montreal Neurological Institute (MNI) scan] in steps of one cm medial, lateral, anterior and posterior. This grid was created using neuronavigation software (Brainsight, Rogue Research Inc, Canada).

At each coordinate, five consecutive stimuli were applied (Cuypers et al., 2014). The position that produced the highest average peak to peak MEP amplitude was chosen to be the hotspot. The coil position, angle and tilt at the hotspot were recorded in the neuronavigation software. In total, 160 pulses were administered. Half of these were given at 90% of the stimulator's maximum intensity, the other 80 pulses were administered at 100%. At both intensities, 40 pulses were administered with a normal current flow (second pulse wave inducing PA current in the cortex) and 40 pulses with reversed current flow (second pulse wave inducing AP current in the cortex).

Main experiment

Dual-site transcranial magnetic stimulation

A dsTMS-paradigm (Ferbert et al., 1992) was employed to probe ipsilateral functional connectivity between left PMd and $M1_{hand}$. Throughout the experiment, participants were asked to take place in a chair and relax. As PMd and $M1_{hand}$ are spatially close to one another (Picard and Strick, 2001), the used dsTMS configuration needed to ensure minimal distance between the two stimulation points. To achieve this, three different configurations were extensively tested using three different types of coils. All coils were manufactured by MagVenture A/S (Farum, Denmark). The first configuration placed a MC-B35 coil over both PMd and $M1_{hand}$. The second setup positioned a MC-B35 coil over PMd and a MC-B70 coil (outer diameter: 97mm) over $M1_{hand}$. The third and final configuration placed a MC-B35 coil over $M1_{hand}$ and the right outer surface of a D-B80 coil (outer diameter: 95mm) over PMd (Fig. 2a). This final configuration was found to be most effective and was thus used in the main experiment. Using this configuration, the distance between the two stimulation sites was approximately 35 mm (Fig. 2b). The MC-B35 coil was attached to a MagPro R30 stimulator (MagVenture A/S, Farum, Denmark). The D-B80 coil was attached to a MagPro X100 stimulator (MagVenture A/S, Farum, Denmark) with MagOption module, enabling the change of current direction in the coil targeting PMd.

Throughout the experiment, the coils were continuously tracked with neuronavigation, showing the position of the coils in relationship to an MRI scan of the participants brain. The anatomical MRI scans will enable us to associate the stimulation effect with the calculated electrical field (e-field) induced in the brain by TMS stimulation. However, since a non-conventional coil configuration (Fig. 2c) was used, a customized and complex adaptation in the e-field software is required. For this, we are dependent on the software developers. Therefore, it was impossible to report the e-field data in the current work.

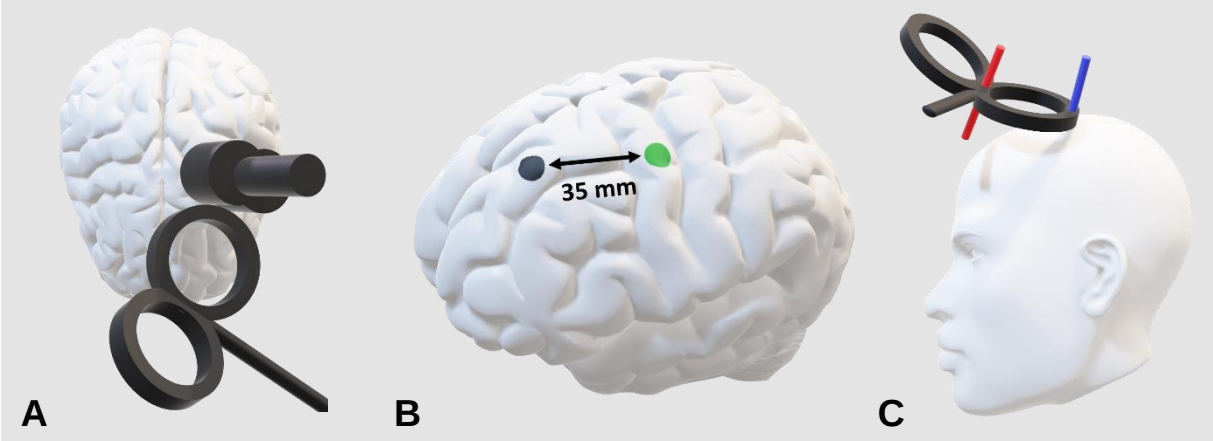


Figure 2. (a) Coil placement in relation to the scalp, anterior coil = D-B80 coil, posterior coil = MC-B35. **(b)** Stimulated areas and interstimulus distance using D-B80 coil targeting left PMd (grey) and MC-B35 coil targeting $M1_{hand}$ (green). **(c)** Orientation of D-B80 coil in relation to the scalp. The red bar illustrates the standard modelling reference point used in software, the blue bar indicates the hypothesized stimulation point used in this study.

The motor representation of the right FDI was determined through a near-identical hotspotting procedure as described in the pilot experiment. The difference being that a grid was projected on the MRI scan of subjects and not on an MNI scan. The stimulator intensity that evoked MEPs with a peak to peak amplitude of approximately 1 mV was determined as well. The D-B80 coil was placed immediately anterior and parallel to the MC-B35 coil targeting $M1_{hand}$ (Groppa et al., 2012a) with the right outer coil surface targetting the presumed left PMd region, as previously described (Fig. 2a). The coil was rotated 180 degrees around the sagittal axis to ensure focal stimulation solely over the PMd area. Alignment of the coil in relation to the MC-B35 coil was done with the goal of minimizing the distance between them and in consideration of previous literature (Groppa et al., 2012a). Finally, the coil position, angle and tilt were recorded by the neuronavigational system. To determine the resting motor threshold (RMT), the right outer surface of the D-B80 coil was placed over $M1_{hand}$. Stimulus intensity was set to the lowest level at which five out of ten pulses over $M1_{hand}$ could still evoke a MEP with a peak to peak amplitude larger than 0.05 mV (Rossini et al., 1994; Groppa et al., 2012a).

Experimental design

The objective of the current study was to determine an effective paradigm investigating PMd - $M1_{hand}$ connectivity. To do so, a conditioning-test approach was used. A TS was given to $M1_{hand}$ (second pulse inducing PA current in the cortex) and a CS to PMd. Two different combinations of intensity and timing were applied (Fig. 3) (Civardi et al., 2001; Groppa et al., 2012a). Each was applied twice, once with normal current direction in the coil targeting PMd (second pulse inducing latero-medial (LM) current in cortex) and once with inversed current direction (second pulse inducing medio-lateral (ML) current in cortex).

Hence, four paradigms were tested: Civardi – normal current (CN), Civardi – reversed current (CR), Groppa – normal current (GN) and Groppa – reversed current (GR). In all paradigms, 35 trials were administered (15 CS + TS, 15 TS and 5 CS trials). TMS pulses had a biphasic configuration since its balanced charge and short pulse duration make it the most suitable choice to research brain connectivity with a dsTMS configuration (Chris and Reza, 2006). All trials were separated by a randomized time interval ranging between 5 and 8 seconds. Before starting, participants were asked to sum up four numbers in a random order. Depending on their answer the order of paradigms was chosen. All TMS pulses within a paradigm were intermixed randomly by Signal software (version 6.05, Cambridge Electronic Design, UK).

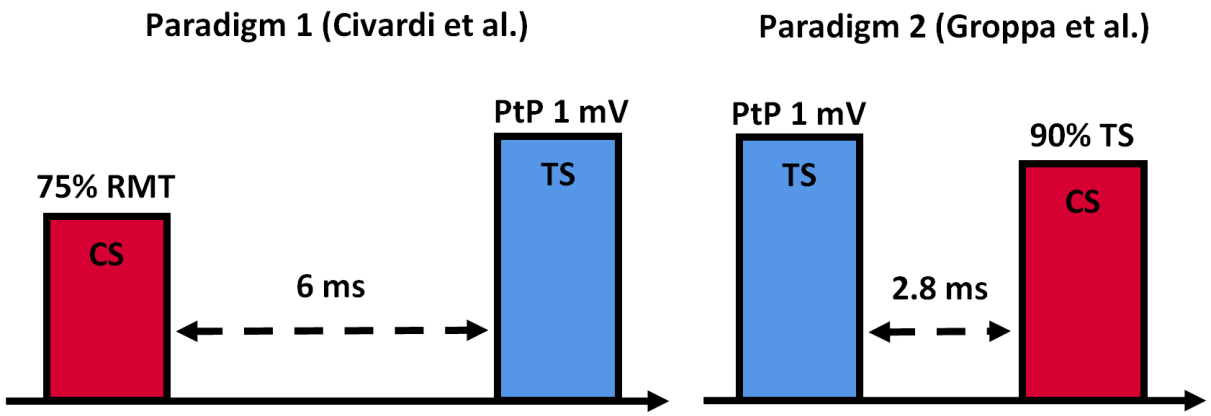


Figure 3. Schematic overview of the two paradigms used in this study, both were applied twice. Once with normal current direction over left PMd and once with inversed current direction. Above the bars, the stimulation intensity is displayed. PtP= peak to peak amplitude

Paradigm I – Civardi et al.

In line with Civardi et al. (2001), the CN and CR conditions had a CS followed by a TS with an ISI of 6ms. TS intensity was adjusted to produce MEPs with a peak to peak amplitude of approximately 1 mV. Although Civardi et al. (2001) used a CS intensity of 90% active motor threshold (AMT), in the current study CS was administered at an intensity of 75% RMT. This was done since direct determination of AMT was not possible, but research found that AMT equals 83% RMT (Cheeran, 2011; Cheeran, 2015). Therefore, assuming the relationship between AMT and RMT is linear, 90% of AMT should equal 75% RMT.

Paradigm II – Groppa et al.

In the GN and GR conditions, TS preceded CS with 2.8 ms, as this ISI induced the largest amount of facilitation (> 25%) in the study from Groppa et al. (2012a). TS was set to an intensity that evoked MEPs with a peak to peak amplitude of approximately 1 mV. CS intensity was equal to 90% TS, in line with the protocol of Groppa et al. (2012a). The number of administered trials was identical to paradigm I (70 pulses in total).

Data analysis

Peak to peak MEP amplitudes were analyzed offline using SPSS 26 (IBM, New York, United States). Data processing and analysis was done respectively with Excel (Microsoft, Washington, United States) and SPSS.

Pilot study

Visual inspection of the Q-Q plot revealed that the pilot data was not normally distributed, this was further confirmed by the Shapiro-Wilk test ($P = 0.011$). Since the assumption of normality was not fulfilled, non-parametric statistical tests were used (Ashby, 1991; Portney and Watkins, 2014). A Wilcoxon Signed Rank Test was conducted to test if MEP peak to peak amplitude distribution differed when using normal versus reversed current direction. This was done for each stimulus intensity and participant.

Main experiment

The data was screened for outliers, with outliers being defined as values deferring from the mean by two SD (Portney and Watkins, 2014). Out of 1260 datapoints, 45 outliers were identified. Since TMS measurements are known to have a high variability (Kiers et al., 1993; Roy Choudhury et al., 2011), it is near impossible to distinguish a valid measurement from an experimental error. In line with this, it was chosen to retain the outliers. All peak to peak MEP amplitudes from the CS + TS condition were divided by the mean MEP amplitude elicited by their counterpart TS condition within the same protocol [(CS + TS) / TS]. This process of data normalization was done in order to reduce data redundancy (Codd, 1990). The mean normalized MEP for each protocol and participant was then calculated, resulting in 36 datapoints [9 subjects x 4 conditions (CN, CR, GN, GR)] used for further analysis.

The visual Q-Q plot and Shapiro-Wilk test ($P < 0.001$) showed that the main experiment dataset was not normally distributed. Non-parametric statistical tests were used (Ashby, 1991; Portney and Watkins, 2014). To analyze which protocol could reliably influence $M1_{hand}$ through PMd, a One-Sample Wilcoxon Signed Rank Test was used. The median normalized MEP of each protocol was compared to the hypothesized median (= 1). The null hypothesis was that there was no difference between the median of CS + TS stimulation and TS only stimulation. Additionally, a Friedman test was conducted to test if there was a significant difference between the four paradigms. Post-hoc analysis of correlations between the four protocols was done using a syntax written in SPSS to enable non-parametric Spearman correlation testing, since this is not a standard feature in SPSS. For all used tests, including those conducted in the pilot study, α was set to 0.05.

RESULTS

Pilot experiment

Table 1 and Figure 4 show the mean MEPs for both participants in all conditions. A Wilcoxon Signed Rank Test was conducted to examine the difference in MEPs evoked by normal (second pulse wave inducing PA current in cortex) versus reversed current stimulation for each participant at both intensities. When stimulating at 90% intensity, there was a significant difference between stimulation with normal versus reversed current direction in both participant one ($P < 0.001$) and two ($P < 0.001$). When changing the intensity to 100%, the difference remained significant in participant one ($P < 0.001$), but not in participant two ($P = 0.192$).

Table 1. Mean Motor Evoked Potentials

Condition	90% NC	90% RC	100% NC	100% RC
Participant 1	0.89 ± 1.12	2.05 ± 0.99	1.64 ± 1.03	2.78 ± 1.06
Participant 2	0.72 ± 0.53	1.03 ± 0.79	3.31 ± 1.26	2.95 ± 1.60

NC= Normal current, RC= Reversed current

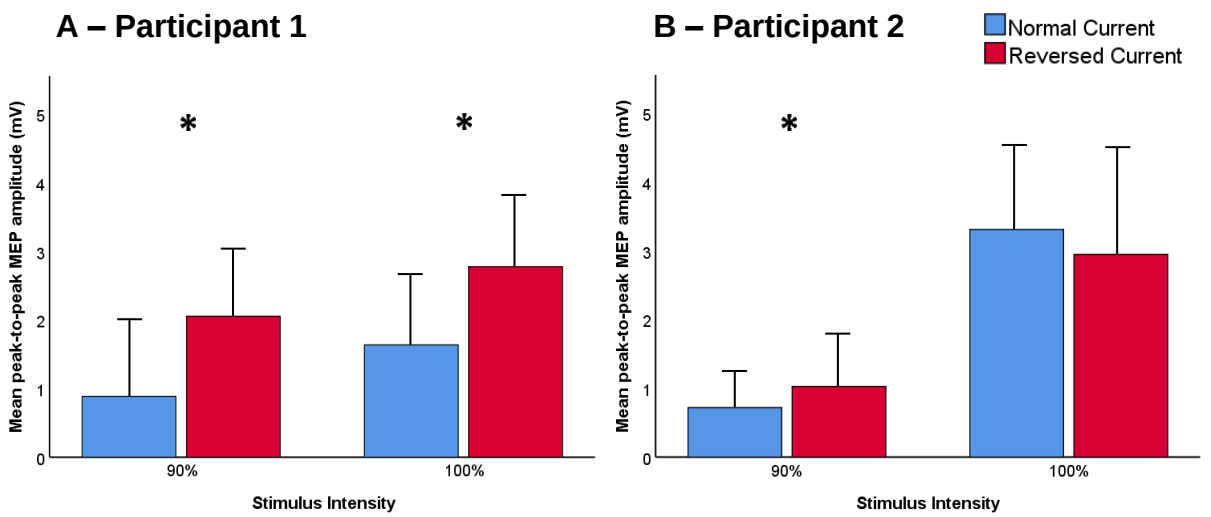


Figure 4. Mean peak to peak MEP amplitude for each stimulation intensity in **(a)** participant 1 and **(b)** 2. The asterisk (*) illustrates a significant difference between stimulation with normal versus reversed current direction within one condition ($P < 0.001$). The whiskers represent standard deviation.

Main experiment

Mean RMT was 75.89% ± 9.45% of maximum stimulator output (D-B80 coil). On average, TS was given at an intensity of 73% ± 12.33% (MC-B35 coil). Mean CS intensity in the CN and CR paradigms was 56.01% ± 6.98%. Mean CS intensity in the GN and GR paradigms was 66.30% ± 11.10%. The procedure was well tolerated by all subjects. Table 2 shows the mean peak to peak MEP amplitudes for each paradigm. Both normalized and raw data are shown.

A One-Sample Wilcoxon Signed Rank Test revealed a significant facilitation effect when using the CN protocol (P = 0.028) (Fig. 5). Peak to peak MEP amplitude in the right FDI increased by 20% in comparison to stimulation over $M1_{hand}$ alone. The other paradigms did not induce a statistically significant effect (CR: P = 0.594, GN: P = 0.678, GR: P = 0.859).

Table 2. Mean MEP amplitudes

Condition	Normalized MEP	TS	TS + CS	CS
CN	**1.20 ± 0.24**	1.37 ± 1.15	1.66 ± 1.39	0.01 ± 0.00
CR	1.05 ± 0.19	1.58 ± 1.29	1.65 ± 1.25	0.01 ± 0.00
GN	1.09 ± 0.28	1.35 ± 1.34	1.41 ± 1.26	0.16 ± 0.50
GR	0.99 ± 0.19	1.53 ± 1.26	1.44 ± 1.27	0.25 ± 0.67
All conditions	1.08 ± 0.66	1.47 ± 1.27	1.54 ± 1.29	0.11 ± 0.43

TS= Test stimulus; CS= Conditioning stimulus; CN= Civardi normal; CR= Civardi reversed; GN= Groppa normal; GR= Groppa reversed. MEPs were normalized through the following calculation: [test stimulus (TS) + conditioning stimulus (CS)] / TS. Values in bold indicate a significant increase in normalized MEP amplitude (P = 0.028).

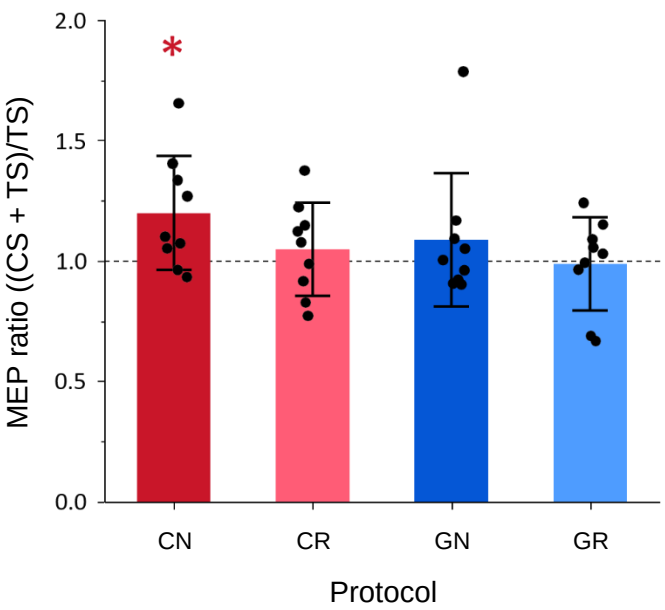


Figure 5. Mean normalized MEPs ± standard deviation (error bars) for each paradigm. CN = Civardi normal; CR = Civardi reversed; GN = Groppa normal; GR = Groppa reversed. Black dots display the individual mean normalized MEP ratio of a subject, calculated with the following formula: [test stimulus (TS) + conditioning stimulus (CS)] / [TS]. The asterisk (*) indicates a significant difference between the [CS + TS] and [TS] condition (P < 0.05).

Figure 6 a – d displays the mean normalized MEP ratio for each paradigm and subject. The Friedman test identified no differences between the normalized MEP distributions of the four paradigms ($\chi^2(3) = 3.000$, $P = 0.392$, Table 2 displays SD & mean values). Non-parametric Spearman correlation testing revealed that there were no correlations between paradigms ($P > 0.05$) (Table 3).

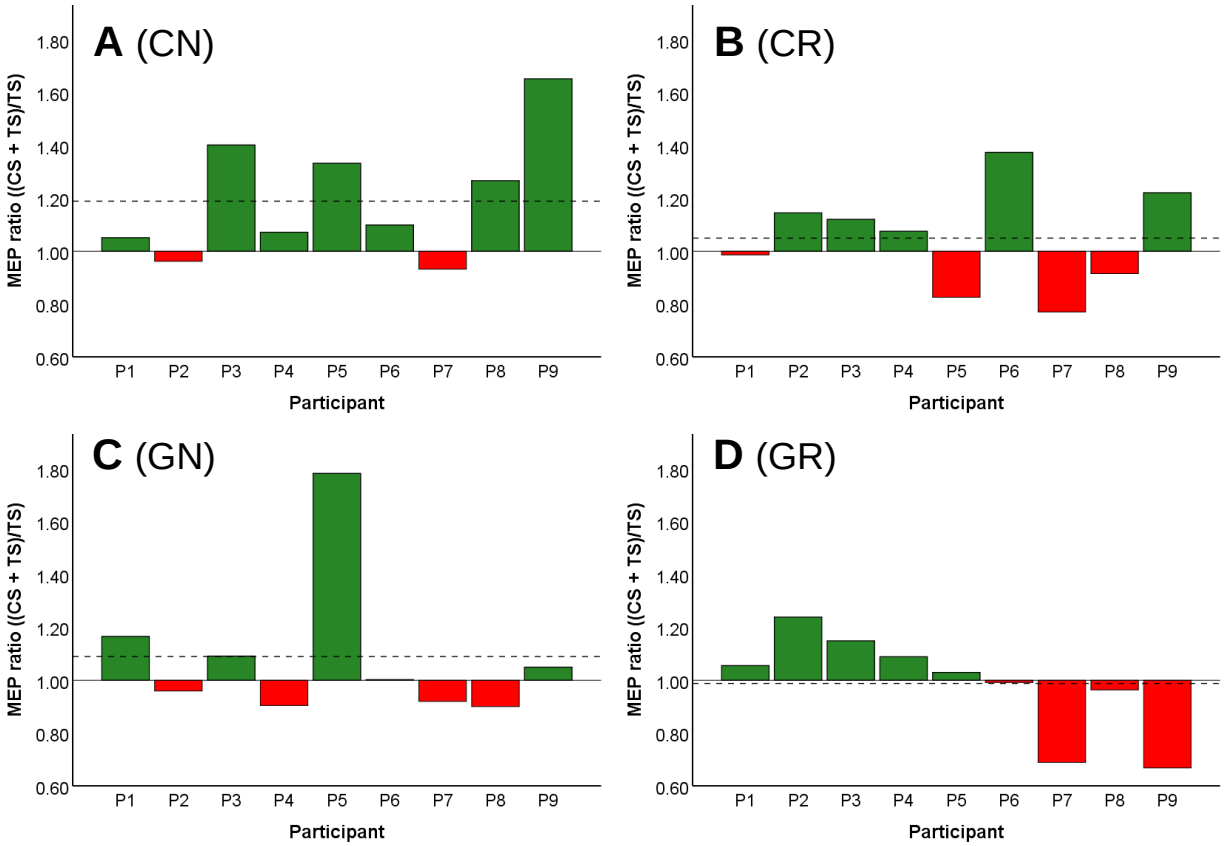


Figure 6. Normalized MEP ratios for each participant (P1 – P9) in paradigm (**a**) CN, (**b**) CR, (**c**) GN and (**d**) GR, calculated with the following formula: [test stimulus (TS) + conditioning stimulus (CS)] / [TS]. MEP ratio = 1 indicates no effect of [CS + TS] in comparison to [TS]. MEP ratio > 1 indicates facilitation (green), MEP ratio < 1 indicates inhibition (red). In each graph, the mean normalized MEP ratio is displayed with a dotted line.

Table 3. Correlations between paradigms

Paradigm		CN	CR	GN	GR
CN	Correlation (ρ)	1.00	0.40	0.13	0.17
	Significance (P)	.	0.33	0.75	0.69
CR	Correlation (ρ)	0.40	1.00	- 0.40	- 0.27
	Significance (P)	0.33	.	0.33	0.51
GN	Correlation (ρ)	0.13	- 0.40	1.00	0.30
	Significance (P)	0.75	0.33	.	0.47
GR	Correlation (ρ)	0.17	- 0.27	0.30	1.00
	Significance (P)	0.69	0.51	0.47	.

CN = Civardi normal; CR = Civardi reversed; GN = Groppa normal; GR = Groppa reversed.

Correlation was calculated through Spearman correlation testing.

DISCUSSION

Pilot experiment

The pilot experiment was conducted to investigate if reversing current direction influences the effect TMS induces, as reported by numerous studies (Civardi et al., 2001; Kammer et al., 2001; Balslev et al., 2007; Hannah and Rothwell, 2017; Vink et al., 2018). To reiterate, in the pilot experiment the current direction was reversed in the coil targeting $M1_{hand}$ while in the main experiment current direction was reversed in the coil targeting PMd. This was done since stimulation over $M1_{hand}$ evokes a clear outcome measure, making it easy to determine if reversing current direction does indeed yield significant importance. TMS over PMd has no clear physiological outcome measure, and therefore cannot verify this as easily (Civardi et al., 2001; Groppa et al., 2012a). As reversing current direction was revealed to yield significant importance, it was opted to implement manipulation of current direction in the main experiment.

Main experiment

The current work demonstrates that when using the CN paradigm, PMd facilitates MEPs evoked in $M1_{hand}$ by 20% on average. This argues in favor of a direct state-dependent cortico-cortical connection between PMd and $M1_{hand}$, as previously hypothesized (Civardi et al., 2001; Groppa et al., 2012a). Since CS alone did not elicit MEPs, both a current spread from the coil targeting PMd to $M1_{hand}$ and a direct premotor-to-muscle pathway can be ruled out as the underlying mechanism. Two implications arise from the current findings

Firstly, the combination of timing and intensity is of significant importance when probing functional connectivity. Results indicate that stimulating PMd 6 ms prior to $M1_{hand}$ with an intensity of 75% RMT (= CN protocol) induces significant results, while protocols based on the study conducted by Groppa et al. (2012a) yield no significant results. With the present knowledge of PMd - $M1_{hand}$ connectivity, it is hard to state a precise explanation as to why this is the case. A possible explanation might be that applying a TMS pulse over PMd influences the generation of I-waves in $M1_{hand}$ only when applied within a narrow time window (Patton and Amassian, 1954; Di Lazzaro et al., 1998; Groppa et al., 2012a). While Groppa et al. (2012a) hypothesized that stimulating PMd after $M1_{hand}$ would still influence the production of late I-waves, the results of the current experiment contradict this. It seems more likely that stimulating PMd 6 ms prior to $M1_{hand}$ can facilitate I-wave generation through pre-excitation of $M1_{hand}$. Several studies have revealed that a TMS pulse over $M1_{hand}$ can modulate I-wave generation caused by a subsequent TMS pulse (Thickbroom et al., 2006; Cash et al., 2009). It's plausible that TMS over PMd can influence I-wave generation in $M1_{hand}$ in a similar manner. However, to this date there is no scientific evidence to substantiate this speculation.

Secondly, the direction of the induced current over PMd significantly affects the influence PMd yields over $M1_{hand}$. To reiterate, PMd was stimulated with a LM and ML current. Due to spatial limitations, these current directions were chosen instead of the conventional PA and AP current directions. Positioning the D-B80 coil in such a manner that a PA or AP current was induced in the cortex while still targeting PMd, was impossible with the current setup. The CN paradigm (second pulse wave inducing LM current in the cortex) was identified as the only paradigm that yielded significant results. When reversing the current direction, no significant effect was found. This infers that TMS inducing a LM current over PMd has a greater faciliatory influence on $M1_{hand}$ then TMS inducing a ML current. This hypothesis is further reinforced by the differences between the GN and GR paradigm. Although nonsignificant, the mean normalized MEPs in the GN paradigm were on average 10% higher than their counterparts in the GR paradigm. The observed discrepancy between stimulation with a LM vs. ML current might be similar to the already investigated discrepancy between AP and PA current stimulation (Di Lazzaro et al., 2001; Hannah and Rothwell, 2017). TMS inducing an AP current in the brain targets later arriving I-waves and possibly even different neuron sites in comparison to a PA current (Di Lazzaro et al., 2001; Di Lazzaro et al., 1998; Hannah and Rothwell, 2017).

Inter-individual variability

In the main experiment, some participants seemed to have a tendency towards facilitation or inhibition regardless of the applied protocol (Fig. 6 a - d). Participant three for example showed facilitation in every paradigm while participant seven showed consistent suppression regardless of the applied protocol. Other participants also displayed a tendency towards facilitation or inhibition, although not as striking. The literature suggests several factors that might play a role in explaining these inter-individual differences in response to TMS.

A first factor is brain derived neurotrophic factor (BDNF). A study from Cheeran et al. (2008) reported that participants respond differently to repetitive TMS depending on the BDNF polymorphism gene they carry. Subjects carrying the Val66Met BDNF gene polymorphism were compared to subjects carrying the Val66Val gene. Results indicated that carriers of the Val66Met gene were less susceptible to the effects of TMS. Similar results were described in a study conducted by Kleim et al. (2006), where single pulse TMS was applied. Possibly, participants in the current study who mostly experienced MEP suppression carried a different BDNF gene then those who experienced facilitation. It is however unlikely that BDNF explains all inter-individual differences found in the current study. Cheeran et al. (2008) and Kleim et al. (2006) reported a reduced or absent effect in individuals that carried the Val66Met gene in comparison to individuals that carried the Val66Val gene, while in the current study suppression and facilitation are described.

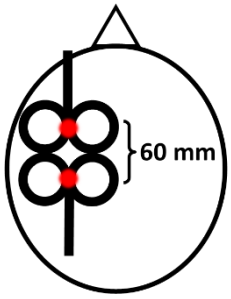
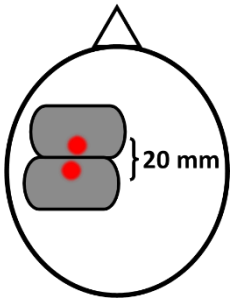
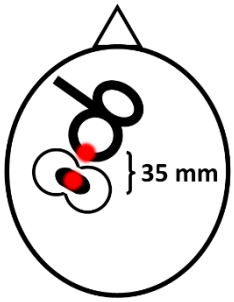
A second factor explaining the inter-individual differences is the placement of the coils. The coil targeting PMd was placed in such a manner that the distance between the two stimulated areas was minimal. Therefore, the area targeted by this coil varied across participants depending on factors such as cortical anatomy and head size. It is thus feasible that different subregions of PMd were targeted (Genon et al., 2018).

A final factor that could partially explain the variability between participants is the strength of the anatomical connections. Boorman et al. (2007) found that variation in white matter integrity between PMd and $M1_{hand}$ is linked to variation in functional connectivity. Hence, differences in white matter integrity might influence the effect PMd yields over $M1_{hand}$.

Discrepancy with previous literature

When comparing the results of the current study with the studies conducted by Civardi et al. (2001) and Groppa et al. (2012a) some inconsistencies stand out. Civardi et al. (2001) reported suppression of MEPs when applying a CS over PMd 6 ms prior to a TS over $M1_{hand}$, while in the current study facilitation was found using mostly the same parameters. Groppa et al. (2012a) noted facilitation when applying CS 2.8 ms after TS whereas in this study no significant effects were found using a similar protocol. These conflicting results can be attributed to several dissimilarities in the applied dsTMS protocol (Table 4). For example, the used coils and their positioning differed. In addition to this, the induced current direction was different across studies as well. Taking into account these discrepancies, it is likely that different subregions of PMd were stimulated (Genon et al., 2018). Stimulation of some subregions might produce facilitation, while other regions might have an inhibitory effect on $M1_{hand}$. This hypothesis is reinforced by the results from Groppa et al. (2012a). These authors found facilitation using a similar interstimulus distance as the current study, in contrast to Civardi et al. (2001) who reported inhibtion. The paradigm used by Civardi et al. (2001) had an interstimulus distance of 60 mm, this makes it reasonable to assume that TMS affected more anterior regions of the medial frontal gyrus and the supplementary motor area instead of PMd (Civardi et al., 2001).

Table 4. Comparison of TMS paradigms

TMS paradigm	Civardi et al. (2001) (n= 11)	Groppa et al. (2012a) (n = 18)	CN paradigm (n = 9)
Interstimulus distance	60 mm	20 mm	35 mm
Coil placement[1]			
Current direction TS	Postero-anterior	Postero-anterior	Postero-anterior
Current direction CS	Antero-posterior	Postero-anterior	Latero-medial
Pulse order	CS → TS	TS → CS	CS → TS
Interstimulus interval	6 ms	2.8 ms	6 ms
PtP evoked by TS	1 mV	0.5 mV	1 mV
CS intensity	90% AMT	90% TS	75% RMT
Effect	Inhibition	Facilitation	Facilitation

[1]Coils in relation to the scalp, the red dot displays the hypothesized stimulation site, TS = Test stimulus, given over primary motor hand area, CS = Conditioning stimulus, given over dorsal premotor cortex, PtP = peak to peak amplitude of MEPs, AMT = active motor threshold, RMT= resting motor threshold

Limitations and implications for future research

Although the present results indicate that the CN paradigm is the most favorable to apply in future studies, some limitations need to be addressed. The current study was considered to be a proof of concept study with a relatively low sample size due to technical and structural limitations. In TMS experiments focusing on a similar topic, on average 15 participants were included (Civardi et al., 2001; Mochizuki et al., 2004; Baumer et al., 2006; Koch et al., 2007; Groppa et al., 2012a; Groppa et al., 2012b; Vesia et al., 2018). Since TMS outcome is highly variable, future studies would benefit strongly from larger population samples (Kiers et al., 1993; Roy Choudhury et al., 2011).

Given that opposed current directions over $M1_{hand}$ modulate this region's plasticity and functional connectivity differently (Day et al., 1989; Huang et al., 2018), it would be interesting for future experiments to change both the current direction over PMd and $M1_{hand}$. In the current study, this was not possible due to only one stimulator having a MagOption module, enabling a reversal of current direction.

Modelling of the current spread in the cortex would also be beneficial to further interpret the present results, as this would enable more precise identification of the brain regions stimulated by TMS. Currently, this was not possible since the modelling reference point of the PMd coil was not touching the skull (Fig. 2c). To overcome this problem, the software developers would need to adapt the e-field software. On a similar note, it is strongly recommended future studies use neuronavigation software to track the location of the coils in relation to the cortex to overcome variability induced by coil positioning.

A final limitation this study was subject to, were the spatial characteristics of the used coils. $M1_{hand}$ was stimulated with the MC-B35 coil, instead of a larger MC-B70 coil, to minimize distance between stimulated areas. Using the smaller coil however had the drawback that four individuals were excluded as TMS could not reliably elicit MEPs in the right FDI. Even when using the MC-B35 coil, the interstimulus distance remained 35 mm. Multi-locus TMS might be the solution to overcome this shortcoming. This relatively new TMS method can theoretically minimize interstimulus distance even further while still having an interstimulus interval in the range of milliseconds (Nieminen et al., 2019). However, in practice multi-locus TMS has not yet been used in dsTMS studies due to financial and technical limitations (Koponen et al., 2018).

CONCLUSION

This proof of concept study demonstrates that investigating intrahemispheric left PMd - $M1_{hand}$ connectivity using a novel dsTMS setup is possible. The implementation of a cooled coil will enable future studies to test for longer periods of time. Significant faciliatory effects were found when delivering a CS to PMd at 75% RMT intensity, 6 ms prior to administrating a TS over $M1_{hand}$ at the 1 mV intensity. Peak to peak MEP amplitude in the right FDI increased by 20% on average. These findings can be a cornerstone for future research focusing on ipsilateral PMd - $M1_{hand}$ interaction during the preparation or execution phase of functional tasks.

REFERENCES

Abe M, Hanakawa T (2009) Functional coupling underlying motor and cognitive functions of the dorsal premotor cortex. Behav Brain Res 198:13-23.

Andrew E (2007) A review of the safety of transcranial magnetic stimulation. In, p 40: Magstim.

Ashby D (1991) Practical statistics for medical research. Douglas G. Altman, Chapman and Hall, London, 1991. No. of pages: 611. Price: £32.00. Statistics in Medicine 10:1635-1636.

Balslev D, Braet W, McAllister C, Miall RC (2007) Inter-individual variability in optimal current direction for transcranial magnetic stimulation of the motor cortex. Journal of Neuroscience Methods 162:309-313.

Baumer T, Bock F, Koch G, Lange R, Rothwell JC, Siebner HR, Munchau A (2006) Magnetic stimulation of human premotor or motor cortex produces interhemispheric facilitation through distinct pathways. J Physiol 572:857-868.

Beets IA, Gooijers J, Boisgontier MP, Pauwels L, Coxon JP, Wittenberg G, Swinnen SP (2015) Reduced Neural Differentiation Between Feedback Conditions After Bimanual Coordination Training with and without Augmented Visual Feedback. Cereb Cortex 25:1958-1969.

Bestmann S, Swayne O, Blankenburg F, Ruff CC, Haggard P, Weiskopf N, Josephs O, Driver J, Rothwell JC, Ward NS (2008) Dorsal premotor cortex exerts state-dependent causal influences on activity in contralateral primary motor and dorsal premotor cortex. Cereb Cortex 18:1281-1291.

Boldrey E, Penfield W (1937) Somatic motor and sensory representation in the cerebral cortex of man as studies by electrical stimulation. Brain 60:389-443.

Bolognini N, Ro T (2010) Transcranial magnetic stimulation: disrupting neural activity to alter and assess brain function. J Neurosci 30:9647-9650.

Boorman ED, O'Shea J, Sebastian C, Rushworth MFS, Johansen-Berg H (2007) Individual Differences in White-Matter Microstructure Reflect Variation in Functional Connectivity during Choice. Current Biology 17:1426-1431.

Cash RFH, Benwell NM, Murray K, Mastaglia FL, Thickbroom GW (2009) Neuromodulation by paired-pulse TMS at an I-wave interval facilitates multiple I-waves. Experimental Brain Research 193:1-7.

Castiello U, Begliomini C (2008) The cortical control of visually guided grasping. Neuroscientist 14:157-170.

Cazzato V (2010) Reflexive Social Attention modulated by Social Cues: evidence from functional Magnetic Resonance Imaging (fMRI) studies. In.

Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards M, Houlden H, Bhatia K, Greenwood R, Rothwell JC (2008) A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. J Physiol 586:5717-5725.

Cheeran, B (2011). Stimulation genomics: probing the effects of genetic variation on human cortical plasticity and its clinical implications.

Cheeran B (2015) What is the best method to measure active motor threshold when calculating TMS intensity in a theta burst protocol? Researchgate available at: https://www.researchgate.net/post/What_is_the_best_method_to_measure_active_motor_threshold_when_calculating_TMS_intensity_in_a_theta_burst_protocol [Accessed March 30, 2020].

Chouinard PA, Paus T (2006) The primary motor and premotor areas of the human cerebral cortex. Neuroscientist 12:143-152.

Chris H, Reza J (2006) The guide to magnetic stimulation. In, p 45: Magstim.

Civardi C, Cantello R, Asselman P, Rothwell JC (2001) Transcranial magnetic stimulation can be used to test connections to primary motor areas from frontal and medial cortex in humans. Neuroimage 14:1444-1453.

Codd EF (1990) The relational model for database management: version 2: Addison-Wesley Longman Publishing Co., Inc.

Cuypers K, Thijs H, Meesen RL (2014) Optimization of the transcranial magnetic stimulation protocol by defining a reliable estimate for corticospinal excitability. PLoS One 9:e86380.

Day BL, Dressler D, Maertens de Noordhout A, Marsden CD, Nakashima K, Rothwell JC, Thompson PD (1989) Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. J Physiol 412:449-473.

Dell'Acqua F, Vergani F, Malik F, Hodge H, Roy P, Valabregue R, Thiebaut de Schotten M (2011) Short frontal lobe connections of the human brain. Cortex; a journal devoted to the study of the nervous system and behavior 48:273-291.

Delvendahl I, Gattinger N, Berger T, Gleich B, Siebner HR, Mall V (2014) The Role of Pulse Shape in Motor Cortex Transcranial Magnetic Stimulation Using Full-Sine Stimuli. PLOS ONE 9:e115247.

Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, Mazzone P, Tonali P, Rothwell JC (1998) Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. Electroencephalogr Clin Neurophysiol 109:397-401.

Di Lazzaro V, Oliviero A Fau - Saturno E, - Pilato F, - Insola A, - Mazzone P, - Profice P, - Tonali P, - Rothwell JC (2001) The effect on corticospinal volleys of reversing the direction of current induced in the motor cortex by transcranial magnetic stimulation.

Di Lazzaro V, Profice P, Ranieri F, Capone F, Dileone M, Oliviero A, Pilato F (2012) I-wave origin and modulation. Brain Stimul 5:512-525.

Dum RP, Strick PL (1991) The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci 11:667-689.

Dum RP, Strick PL (2005) Frontal lobe inputs to the digit representations of the motor areas on the lateral surface of the hemisphere. J Neurosci 25:1375-1386.

Duque J, Labruna L, Verset S, Olivier E, Ivry RB (2012) Dissociating the role of prefrontal and premotor cortices in controlling inhibitory mechanisms during motor preparation. J Neurosci 32:806-816.

Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG, Marsden CD (1992) Interhemispheric inhibition of the human motor cortex. J Physiol 453:525-546.

Fiori F, Chiappini E, Candidi M, Romei V, Borgomaneri S, Avenanti A. Long-latency interhemispheric interactions between motor-related areas and the primary motor cortex: a dual site TMS study. Sci Rep (2017) 7(1):14936. doi:10.1038/s41598-017-13708-2

Fricke C, Duesmann C, Woost TB, von Hofen-Hohloch J, Rumpf J-J, Weise D, Classen J (2019) Dual-Site Transcranial Magnetic Stimulation for the Treatment of Parkinson's Disease. Frontiers in Neurology 10.

Fujiyama H, Van Soom J, Rens G, Cuypers K, Heise KF, Levin O, Swinnen SP (2016) Performing two different actions simultaneously: The critical role of interhemispheric interactions during the preparation of bimanual movement. Cortex 77:141-154.

Genon S, Li H, Fan L, Müller VI, Cieslik EC, Hoffstaedter F, Reid AT, Langner R, Grefkes C, Fox PT, Moebus S, Caspers S, Amunts K, Jiang T, Eickhoff SB (2016) The Right Dorsal Premotor Mosaic: Organization, Functions, and Connectivity. Cerebral Cortex 27:2095-2110.

Genon S, Reid A, Li H, Fan L, Muller VI, Cieslik EC, Hoffstaedter F, Langner R, Grefkes C, Laird AR, Fox PT, Jiang T, Amunts K, Eickhoff SB (2018) The heterogeneity of the left dorsal premotor cortex evidenced by multimodal connectivity-based parcellation and functional characterization. Neuroimage 170:400-411.

Glover GH (2011) Overview of functional magnetic resonance imaging. Neurosurg Clin N Am 22:133-139, vii.

Groppa S, Schlaak BH, Munchau A, Werner-Petroll N, Dunnweber J, Baumer T, van Nuenen BF, Siebner HR (2012a) The human dorsal premotor cortex facilitates the excitability of ipsilateral primary motor cortex via a short latency cortico-cortical route. Hum Brain Mapp 33:419-430.

Groppa S, Werner-Petroll N, Munchau A, Deuschl G, Ruschworth MF, Siebner HR (2012b) A novel dual-site transcranial magnetic stimulation paradigm to probe fast facilitatory inputs from ipsilateral dorsal premotor cortex to primary motor cortex. Neuroimage 62:500-509.

Guye M, Parker GJ, Symms M, Boulby P, Wheeler-Kingshott CA, Salek-Haddadi A, Barker GJ, Duncan JS (2003) Combined functional MRI and tractography to demonstrate the connectivity of the human primary motor cortex in vivo. Neuroimage 19:1349-1360.

Hannah R, Rothwell JC (2017) Pulse Duration as Well as Current Direction Determines the Specificity of Transcranial Magnetic Stimulation of Motor Cortex during Contraction. Brain Stimulation 10:106-115.

He SQ, Dum RP, Strick PL (1993) Topographic organization of corticospinal projections from the frontal lobe: motor areas on the lateral surface of the hemisphere. J Neurosci 13:952-980.

Hlustik P, Solodkin A, Gullapalli RP, Noll DC, Small SL (2001) Somatotopy in human primary motor and somatosensory hand representations revisited. Cereb Cortex 11:312-321.

Huang Y-Z, Chen R-S, Fong P-Y, Rothwell JC, Chuang W-L, Weng Y-H, Lin W-Y, Lu C-S (2018) Inter-cortical modulation from premotor to motor plasticity. The Journal of Physiology 596:4207-4217.

Janssen AM, Oostendorp TF, Stegeman DF (2015) The coil orientation dependency of the electric field induced by TMS for M1 and other brain areas. J Neuroeng Rehabil 12:47.

Kammer T, Beck S, Erb M, Grodd W (2001) The influence of current direction on phosphene thresholds evoked by transcranial magnetic stimulation. Clinical Neurophysiology 112:2015-2021.

Kiers L, Cros D, Chiappa KH, Fang J (1993) Variability of motor potentials evoked by transcranial magnetic stimulation. Electroencephalography and clinical neurophysiology 89:415-423.

Kleim JA, Chan S, Pringle E, Schallert K, Procaccio V, Jimenez R, Cramer SC (2006) BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. In: Nat Neurosci, pp 735-737. United States.

Koch G, Franca M, Mochizuki H, Marconi B, Caltagirone C, Rothwell JC (2007) Interactions between pairs of transcranial magnetic stimuli over the human left dorsal premotor cortex differ from those seen in primary motor cortex. J Physiol 578:551-562.

Koponen LM, Nieminen JO, Ilmoniemi RJ (2018) Multi-locus transcranial magnetic stimulation—theory and implementation. Brain Stimulation 11:849-855.

Lemon RN (2008) Descending pathways in motor control. Annu Rev Neurosci 31:195-218.

Mochizuki H, Huang Y-Z, Rothwell JC (2004) Interhemispheric interaction between human dorsal premotor and contralateral primary motor cortex. The Journal of Physiology 561:331-338.

Moisa M, Siebner HR, Pohmann R, Thielscher A (2012) Uncovering a Context-Specific Connectional Fingerprint of Human Dorsal Premotor Cortex. The Journal of Neuroscience 32:7244-7252.

Niemann N, Wiegel P, Kurz A, Rothwell JC, Leukel C (2018) Assessing TMS-induced D and I waves with spinal H-reflexes. Journal of neurophysiology 119:933-943.

Nieminen JO, Koponen LM, Mäkelä N, Souza VH, Stenroos M, Ilmoniemi RJ (2019) Short-interval intracortical inhibition in human primary motor cortex: A multi-locus transcranial magnetic stimulation study. NeuroImage 203:116194.

Ni Z, Isayama R, Castillo G, Gunraj C, Saha U, Chen R. Reduced dorsal premotor cortex and primary motor cortex connectivity in older adults. Neurobiol Aging (2015) 36(1):301–3. doi:10.1016/j.neurobiolaging.2014.08.017

O'Shea J, Sebastian C, Boorman ED, Johansen-Berg H, Rushworth MF (2007) Functional specificity of human premotor-motor cortical interactions during action selection. Eur J Neurosci 26:2085-2095.

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97-113.

Patton HD, Amassian VE (1954) Single and multiple-unit analysis of cortical stage of pyramidal tract activation. J Neurophysiol 17:345-363.

Picard N, Strick PL (2001) Imaging the premotor areas. Curr Opin Neurobiol 11:663-672.

Portney LG, Watkins MP (2014) Foundations of clinical research: applications to practice, 3rd ed., new int. ed. Edition: Harlow : Pearson.

Ramsey JD, Hanson SJ, Hanson C, Halchenko YO, Poldrack RA, Glymour C (2010) Six problems for causal inference from fMRI. NeuroImage 49:1545-1558.

Raos V, Umilta MA, Murata A, Fogassi L, Gallese V (2006) Functional properties of grasping-related neurons in the ventral premotor area F5 of the macaque monkey. J Neurophysiol 95:709-729.

Rizzo V, Siebner HR, Modugno N, Pesenti A, Münchau A, Gerschlager W, Webb RM, Rothwell JC (2004) Shaping the excitability of human motor cortex with premotor rTMS. The Journal of Physiology 554:483-495.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A (2009) Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 120:2008-2039.

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijević MR, Hallett M, Katayama Y, Lücking CH, et al. (1994) Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalography and clinical neurophysiology 91:79-92.

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P. B., George, M. S., Hallett, M., Lefaucheur, J. P., Langguth, B., Matsumoto, H., Miniussi, C., Nitsche, M. A., Pascual-Leone, A., Paulus, W., Rossi, S., Rothwell, J. C., … Ziemann, U. (2015) Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol 126:1071-1107.

Roy Choudhury K, Boyle L, Burke M, Lombard W, Ryan S, McNamara B (2011) Intra subject variation and correlation of motor potentials evoked by transcranial magnetic stimulation. Irish journal of medical science 180:873-880.

Ruff CC, Driver J, Bestmann S (2009) Combining TMS and fMRI: from 'virtual lesions' to functional-network accounts of cognition. Cortex 45:1043-1049.

Sliwinska MW, Vitello S, Devlin JT (2014) Transcranial magnetic stimulation for investigating causal brain-behavioral relationships and their time course. J Vis Exp.

Terao Y, Ugawa Y (2002) Basic mechanisms of TMS. Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society 19:322-343.

Toga, A. W. (2015). Brain mapping: An encyclopedic reference.

Thickbroom GW, Byrnes ML, Edwards DJ, Mastaglia FL (2006) Repetitive paired-pulse TMS at I-wave periodicity markedly increases corticospinal excitability: A new technique for modulating synaptic plasticity. Clinical Neurophysiology 117:61-66.

Vesia M, Culham JC, Jegatheeswaran G, Isayama R, Le A, Davare M, Chen R (2018) Functional interaction between human dorsal premotor cortex and the ipsilateral primary motor cortex for grasp plans: a dual-site TMS study. Neuroreport 29:1355-1359.

Vink JJ, Petrov PI, Mandija S, Dijkhuizen RM, Neggers SF (2018) Outcome of TMS-based motor mapping depends on TMS current direction. In. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Wagner T, Valero-Cabre A, Pascual-Leone A (2007) Noninvasive human brain stimulation. Annu Rev Biomed Eng 9:527-565.

Wassermann EM (1998) Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. Electroencephalogr Clin Neurophysiol 108:1-16.