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Calvert, G. H. M., McMackin, R., & Carson, R. G. (2020). Probing interhemispheric dorsal premotor-primary motor cortex interactions with threshold hunting transcranial magnetic stimulation. *Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology*, 131(11), 2551-2560. https://doi.org/10.1016/j.clinph.2020.07.020

**Published in:**
*Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology*

**Document Version:**
Publisher's PDF, also known as Version of record

**Queen's University Belfast - Research Portal:**
Link to publication record in Queen's University Belfast Research Portal

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Probing interhemispheric dorsal premotor-primary motor cortex interactions with threshold hunting transcranial magnetic stimulation

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A R T I C L E   I N F O

Article history:
Accepted 14 July 2020
Available online 26 August 2020

Keywords:
Adaptive threshold hunting
Interhemispheric inhibition
Upper limb
Paired pulse
TMS
PEST

HIGHLIGHTS

- PMd-M1 interhemispheric inhibition (IHI) is seen with PA but not AP test stimuli.
- PMd-M1 IHI increased as a function of CS intensity but did not vary by ISI.
- M1-M1 IHI increased with CS intensity for test stimuli that induced PA current flow.

A B S T R A C T

Objective: To characterise the effect of altering transcranial magnetic stimulation parameters on the magnitude of interhemispheric inhibition (IHI) from dorsal premotor (PMd) to primary motor cortex (M1).

Method: We used a fully automated adaptive threshold hunting paradigm to quantify PMd-M1 IHI across a range of conditioning stimulus (CS) intensities (90%, 110%, 130% of resting motor threshold, rMT) and interstimulus intervals (ISIs) (8, 10, 40 ms). M1-M1 IHI was examined with CS intensities of 110%, 120%, and 130% rMT and ISIs of 10 and 40 ms. Two test coil orientations (inducing posterior-anterior or anterior-posterior current) were used.

Results: PMd-M1 IHI was obtained consistently with posterior-anterior (but not anterior-posterior) test stimuli and increased with CS intensity. M1-M1 IHI was expressed across all conditions and increased with CS intensity when posterior-anterior but not anterior-posterior induced current was used.

Conclusions: The expression of PMd-M1 IHI is contingent on test coil orientation (requiring posterior-anterior induced current) and increases as a function of CS intensity. The expression of M1-M1 IHI is not dependent on test coil orientation.

Significance: We defined a range of parameters that elicit reliable PMd-M1 IHI. This (threshold hunting) methodology may provide a means to quantify premotor-motor pathology and reveal novel quantitative biomarkers.

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1. Introduction

1.1. The clinical significance of callosal integrity

Callosal pathology has been implicated in a number of neurodegenerative conditions, such as amyotrophic lateral sclerosis (Bede and Hardiman, 2017; Dukic et al., 2019), classical progressive supranuclear palsy (Lenka et al., 2017), and both pre- and post-symptomatic Huntington’s disease (Phillips et al., 2013). In respect of these conditions, there exists an urgent need to develop quantitative, affordable biomarkers, in order to improve clinical trial sensitivity and to equip clinicians with early diagnostic tools. Consequently, the establishment of a reliable methodology with which to quantify the functional integrity of callosal projections, which does not depend upon costly neural imaging, is likely to be of practical utility in both clinical and basic scientific contexts.

1.2. Quantifying interhemispheric interactions between motor areas

Dual-site transcranial magnetic stimulation has been applied widely with the aim of assaying interactions between the two primary motor cortices (M1). Inter-hemispheric inhibition (IHI) refers to the decrease in the amplitude of a “test” motor evoked potential (MEP) that is obtained in response to stimulation delivered over...
M1, in circumstances in which an initial “conditioning” stimulus (CS) has been applied 6–15 ms previously to the opposite M1 (Ferbert et al., 1992). Direct callosal projections between left and right caudal M1, i.e. the cortical zones from which the majority of fast-conducting corticospinal axons that innervate spinal motoneurons arise, are however relatively sparse (Ruddy et al., 2017). An argument can therefore be made that in applying electrophysiological techniques for diagnostic/prognostic purposes, the focus should instead be upon inter-hemispheric projections that, on anatomical grounds at least, are more likely to be implicated in integrative functions.

In this respect, interhemispheric projections arising from dorsal premotor cortex (PMd) are of particular interest. PMd exhibits rich connectivity with both the caudal and rostral M1 of the opposite hemisphere, via callosal projections that are characterised by high fibre density (Ruddy et al., 2017). The region also has well documented involvement in motor pathology (Bestmann et al., 2010; Liao et al., 2019). In principle, the functional contributions of interhemispheric projections from PMd may be probed using TMS to derive electrophysiological measures such as IHI (Fiori et al., 2017; Mochizuki et al., 2004). With respect to PMd TMS conditioning however, only a limited range of “peri-threshold” intensities (90–110% rMT) and inter-stimulus intervals (ISIs) have thus far been investigated. A central objective of the present study was to define a range of stimulation parameters that can be used to elicit reliable PMd-M1 IHI.

1.3. The effect of stimulating coil orientation

When TMS is applied to M1, the magnetic stimulating coil is most frequently oriented such that the current induced in the brain moves in a latero-posterior to antero-medial (termed the posterior-anterior [PA] direction. At least partially distinct populations of fibers are excited when the test coil is instead oriented to induce anterior-posterior (AP) directed currents (Di Lazzaro et al., 2001). It is thought that the magnitude of late indirect (e.g. I3) waves generated by TMS, which are preferentially elicited by AP stimuli (Sakai et al., 1997), may reflect the state of excitation of pyramidal neurons with cells bodies in premotor cortex on the crown of the precentral gyrus, or somatosensory cortex on the crown of the postcentral gyrus, which project onto layer 5 cells in M1 via long range corticocortical fibers (Laakso et al., 2013; Triesch et al., 2015). These afferent fibres, which constitute the main cortical input to M1 (DeFelipe et al., 1986; Jones, 1983), may be especially sensitive to AP currents (Esser et al., 2005; Laakso et al., 2013). Although callosal fibres are the epitome of long-range corticocortical projections, they ramify in multiple layers (Cissé et al., 2003). It is thus challenging to predict on theoretical grounds alone whether the effects of input to M1 invoked by CS applied to the opposite PMd, will be revealed most clearly by test stimuli that induce PA or AP currents. A second objective of the study therefore was to assess the impact of test stimulus coil orientation (i.e. inducing PA or AP current) on the magnitude of PMd-M1 IHI.

1.4. Adaptive threshold hunting

Adaptive threshold hunting (ATH), which is sometimes referred to as threshold tracking TMS (Cirillo and Byblow, 2016; Vucic et al., 2018), is a method that incorporates maximum likelihood estimation to “maintain constant” the amplitude of the test MEP (the “threshold hunting target”, THT) by adjusting the TS intensity online during the stimulation protocol (Awiszus et al., 1999). Consequently, IHI is defined as the percentage change in stimulator output necessary to evoke a response of THT amplitude (i.e. in the presence of conditioning), rather than as the percentage change in MEP amplitude at some fixed TS intensity (see Fig. 1C & 1D). The advantages of ATH are that: (1) by maintaining fixed the amplitude of the test response, the spinal and peripheral (Fisher et al., 2002) and central (Opie and Semmler, 2014) mechanisms that mediate the expression of MEPs exert a relatively consistent influence; and (2), if the THT lies on the appropriate portion of the nonlinear TMS stimulus-MEP response curve, the method is thought to reduce the influence of the trial-to-trial variability that is an inherent characteristic of magnetically evoked motor responses (Vucic et al., 2006). In the present study, we employed a fully automated ATH protocol to examine PMd-M1 IHI across a range of TS intensities (90, 110, 130% rMT) and interstimulus intervals (8, 10, 40 ms).

2. Methods

2.1. Participants

Thirty-two neurologically healthy volunteers (15 females, mean age = 24.6, sd = 5.7 years) each participated in a single experiment. None reported a history of neurological or psychiatric disease, or any medication use for which TMS was contraindicated (Rossi et al., 2011, 2009). All participants were right-handed as per the Edinburgh Handedness Inventory (Oldfield, 1971) and provided written informed consent to procedures approved by the Faculty of Engineering and Physical Sciences Research Ethics Committee at Queen’s University Belfast. With the exception of study preregistration, all procedures were conducted in accordance with the Declaration of Helsinki.

2.2. Electromyography

Electromyographic (EMG) activity was recorded from left and right extensor carpi radialis longus (ECR) using pairs of Ag-AgCl electrodes (Cleartrace 1700, ConMed, Haverhill, MA, USA). A reference electrode was placed on the lateral epidymoid of the right humerus. As part of the procedures for determining the position of the right PMd hotspot (see Section 2.3, ‘‘Transcranial Magnetic Stimulation’’), EMG activity was also recorded from the left first dorsal interosseous (FDI) muscle using a pair of 10 mm Ag-AgCl cup electrodes (Viasys Healthcare, Old Woking, UK) impregnated with an electroconductive paste (EC2, Grass Technologies, West Warwick, RI, USA). The skin overlaying the electrode sites was prepared with a topical abrasive (Nuprep, Weaver & Co., Aurora, CO, USA) and sanitised thereafter with a topical antiseptic (70% isopropanol). EMG signals were amplified (gain = 1000), band-pass filtered (30–1000 Hz) and notch filtered (50 Hz) (NL824 & NL820, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK), digitized at a sampling rate of 10 kHz (Power1401, CED, Cambridge, UK), and recorded with Signal software (Signal 7.01, CED, Cambridge, UK).

2.3. Transcranial magnetic stimulation

Monophasic magnetic stimuli were delivered via a pair of Magstim 200 stimulators (Magstim Company Ltd., Whitland, UK), each equipped with a 55 mm mid-diameter figure-of-eight coil. The optimal position (“hotspot”) to elicit a MEP in the left and right ECR and left FDI muscles was first determined. The procedure commenced by moving the coil in ~ 0.5 cm increments about a craniometrically defined location (2 x 6 cm anterolateral to the vertex). Thereafter, the hotspot was defined as the position over the scalp from which MEPs were elicited most reliably in the target muscle of the contralateral limb. The axis of intersection between the two loops was oriented at 45° to the sagittal plane to induce PA current flow across the motor strip of left M1, or at 90° to the sagittal plane.
to induce lateromedial (LM) current flow across right M1 (see Fig. 1). The hotspot was registered in three-dimensional space using a neuro-navigation system (Brainsight, Rogue Resolutions Ltd., Cardiff, UK). PMd was defined craniometrically as the region of scalp situated 2.5 cm anteromedial to the FDI hotspot (i.e. of right M1) – obtained using the coil orientation that induced PA current flow (Mochizuki et al., 2004). Once defined, the PMd hotspot was also registered using the Brainsight system.

Resting motor thresholds (rMT) were obtained using a maximum likelihood protocol – namely parameter estimation by sequential testing (PEST) (Awiszus and Borckardt, 2011), to determine the minimum stimulation intensity required to elicit a MEP with a peak-to-peak amplitude of 50 µV in the contralateral ECR muscle (Rossi et al., 2009). The PEST procedure, which is identical to that incorporated in the ATH protocol, utilises a sigmoid-shaped logistic function to determine the stimulation intensity at which there exists a 0.5 probability of eliciting a MEP with the peak-to-peak amplitude that has been defined (i.e. 50 µV). This function and its implementation in the commonly used MTAT 2.0 programme are described elsewhere by its author (Awiszus, 2003).

The use of the PEST algorithm for the purposes of threshold hunting has been reported previously. To facilitate ease of use, and to reduce the potential for human error that is an inherent risk of manual implementations, we established (with assistance and expertise from Prof. Friedemann Awiszus, see Awiszus et al., 1999) a fully automated procedure in which an interface between SIGNAL (CED Ltd., Cambridge UK) and MATLAB (MathWorks Inc., MA, USA) scripts was used to track EMG output, update stimulator output and calculate threshold intensities online during data collection. In brief, peak-to-peak MEP amplitudes were calculated immediately after each MEP was sampled. These values were then passed to a PEST algorithm to determine the next stimulation intensity (to which the stimulator was then set using the Magstim 200 digital control protocol). The experimenters were required only to hold the coil in position (using Brainsight guidance) as 20 pulses were delivered every 5 s (jittered ± 500 ms), after which a threshold stimulus intensity was determined (i.e. rMT/THT).

For left M1, the rMT was determined twice – once with the test coil oriented to induce AP current flow, and once with the test coil oriented to induce current flow in the PA direction. The minimum stimulation intensity required to elicit the THT (i.e. in this case defined as a MEP of 200 µV amplitude in right ECR) was then also determined for each of the two coil orientations. For right M1, the rMT was determined with the conditioning coil oriented to induce LM current flow across the motor strip (see Fig. 1A and 1B).

2.4. Interhemispheric inhibition

The CS was applied to right M1 or right PMd. The conditioning coil was oriented 90° to the sagittal plane to induce LM current.
flow. The test coil orientation was varied by block to induce current flow in either the AP or PA directions (see Fig. 1A and 1B). The TS intensity was adjusted online using the ATH protocol described above, in order to elicit a MEP with a peak-to-peak amplitude of 200 μV (i.e. the THT). During each block, a TS with an intensity equivalent to that necessary to elicit the THT was delivered during the fifth trial in the absence of a CS, with the view of monitoring the consistency of the THT response, i.e. relative to the expected amplitude of 200 μV.

In assessing IHI between the primary motor cortices (M1-M1 IHI), the CS intensity was set to 110%, 120%, or 130% rMT. The interstimulus interval (ISI, i.e. separating the CS and TS) was 10 or 40 ms. The combination of three CS intensities (110%, 120%, 130% rMT), two ISIs (10, 40 ms), and two TS orientations (AP, PA) gave rise to a total of 12 unique M1-M1 IHI conditions. IHI between right PMd and left M1 (PMd-M1 IHI) was assessed with CS intensities of 90%, 110%, or 130% rMT and ISIs of 8, 10, or 40 ms. The combination of three CS intensities (90%, 110%, 130% rMT), three ISIs (8, 10, 40 ms), and two TS orientations (AP, PA) yielded a total of 18 unique PMd-M1 IHI conditions.

2.5. General procedures

Participants were seated in a comfortable chair with their upper limbs supported, the forearms in mid-pronation and the elbows semi-flexed (100–120°). There were four blocks of stimulation (M1-M1AP, M1-M1PA, PMd-M1AP, PMd-M1PA), each separated by 5 min rest intervals. Only a single TS orientation (AP, PA) was employed in each block. The block orders were counterbalanced and pseudo-randomised across participants. The order in which each condition (i.e. a combination of CS intensity and ISI) was presented within each block was pseudo-randomised. The interval between the completion of the trials in one condition, and the commencement of trials in the following condition, was approximately 2 minutes. The duration of each block was ~43 and ~28 min for the PMd-M1 and M1-M1 IHI blocks respectively.

As a means of eliminating instances in which elevated excitability of the spinal motoneuron pool may have augmented MEP amplitude, we calculated online (i.e. for the target muscle) the root mean square (rms) of the background EMG in the interval 90 ms prior to the CS onset. In the case of estimating the rMT or THT, the interval prior to the TS was used instead. If the rms value exceeded 5 μV, the corresponding MEP was disregarded from the PEST procedure and the trial repeated until such time that the background EMG fell within the permitted limits (rms < 5 μV). Due to the extended duration of the testing session (>3 hours), not all participants completed all conditions (i.e. the time allocated for testing was in some cases reached before the completion of all four blocks). The number of participants that completed each condition is reported in Tables 1–4.

2.6. Data analysis

Interhemispheric inhibition (IHI) was defined as the change in test stimulator output necessary to evoke a MEP of THT amplitude (200 μV), expressed as a percentage of the non-conditioned THT intensity:

\[
IHI(\%) = \left( \frac{THT_{\text{cond}} - THT_{\text{non-cond}}}{THT_{\text{non-cond}}} \right) \times 100
\]

where larger positive values are indicative of greater inhibition.

It is widely recognised (Abelson, 2012) that in repeated measures designs, inferential tests which rely upon standard normal theory often fail to satisfy several key assumptions, including normality of the sample distribution. When assessed using the Shapiro-Wilk test, 43.3% (13/30) of cells failed to satisfy the conventional criterion of asymptotic normality (p < 0.05). Accordingly, we employed robust non-parametric alternatives to repeated-measures factorial ANOVAs, which minimise the potential influence of both outliers and deviations from normality, as the basis of our inferential analyses using the nparLD package in R (Noguchi et al., 2012). These models, in which CS intensity and ISI were fixed effects, were computed separately for each block. In the event of a significant main effect and/or interaction arising, post-hoc analyses based on Yuen's t-tests (trimming interval = 0.2) were computed. In addition, a series of one-sample 95% percentile bootstraps (10,000 samples, μ = 0) – in which Huber's one-step M-estimator (Ψ) was used as a robust measure of location, were computed to verify that IHI was present in each condition, using the WRS2 package in R (Mair and Wilcox, 2016). In seeking to control for multiplicity, adjusted p-values were obtained using the Hochberg step-up procedure (Hochberg, 1988). To assist in the interpretation of the tests of significance, the robust variant of Cohen's d (d̂) and the corresponding bias-corrected-and-accelerated (BCa) bootstrapped 95% confidence interval (10,000 samples) were computed. To ensure that the control MEP amplitudes varied about the expected THT amplitude (i.e. 200 μV), robust tests of equivalence were also undertaken.

3. Results

3.1. PMd-M1 interhemispheric inhibition (PA test stimuli)

PMd-M1 IHI with the test coil oriented to induce PA current flow was reliably expressed across most conditions, with the exception of the "8 ms ISI, 90% rMT CS" and "10 ms ISI, 110% rMT CS" conditions (Table 1 and Fig. 2a). A robust rank-based non-parametric model with CS intensity (levels = 90%, 110%, 130% rMT) and ISI (levels = 8 ms, 10 ms, 40 ms) as fixed effects revealed a main effect of CS intensity (F (1,83, ∞) = 5.49, p = 0.005). There was no effect of ISI (p = 0.710) or a CS intensity × ISI interaction (p = 0.535). Post-hoc Hochberg-corrected Yuen’s t-tests revealed that the magnitude of PMd-M1 IHI (elicited with PA test stimuli) tended to increase as a function of CS intensity. Specifically, we found that the magnitude of PMd-M1 IHI elicited with a CS intensity of 130% rMT was significantly greater than that elicited by a CS intensity of 90% rMT (t (13) = 3.00, p_corrected = 0.031, d̂ = 0.84 (0.40, 1.64)). All other contrasts in which PMd-M1 IHI was compared across CS intensities (i.e. 130% rMT vs. 110% rMT, and 110% rMT vs. 90% rMT) failed to achieve conventional levels of statistical significance (all p_corrected > 0.05).

3.2. PMd-M1 interhemispheric inhibition (AP test stimuli)

PMd-M1 IHI was not expressed reliably (all p_corrected > 0.05) in circumstances in which the test coil was oriented to induce current flow in the AP direction (Table 2 and Fig. 2b).

3.3. M1-M1 interhemispheric inhibition (PA test stimuli)

M1-M1 IHI with the test coil oriented to induce PA current flow was reliably expressed across all conditions (Table 3 and Fig. 3a). A robust rank-based non-parametric model with CS intensity (levels = 110%, 120%, 130% rMT) and ISI (levels = 10 ms, 40 ms) as fixed effects revealed a main effect of CS intensity (F (1,89, ∞) = 4.55, p = 0.012). There was no effect of ISI (p = 0.26) or a CS intensity × ISI interaction (p = 0.638). Post-hoc Hochberg-corrected Yuen’s t-tests indicated that the magnitude of M1-M1
The relevant (adjusted) p-values ($p_{\text{corrected}}$), sample sizes ($n$), and effect size estimates (Cohen’s $d$) – the latter alongside the corresponding BCa bootstrapped 95% confidence interval (10,000 samples), are also provided.

### Table 1

| ISI   | CS/rMT | $n$ | IHI (% 95% CI)/% | $p_{\text{corrected}}$ | Cohen’s $d$ (95% BCA CI) |
|-------|--------|-----|-----------------|-------------------------|-------------------------|
| 8 ms  | 90%    | 31  | 2.77 (0.92, 3.30) | 0.257                   | 0.43 (0.12, 0.94)        |
|       | 110%   | 28  | 5.59 (2.12, 9.49) | 0.025                   | 0.62 (0.27, 1.07)        |
|       | 130%   | 29  | 10.00 (5.09, 15.02) | 0.004*                  | 0.71 (0.27, 1.27)        |
| 10 ms | 90%    | 30  | 4.92 (2.12, 5.84) | 0.016*                  | 0.74 (0.32, 1.26)        |
|       | 110%   | 30  | 4.68 (1.03, 8.51) | 0.143                   | 0.47 (0.11, 0.939)       |
|       | 130%   | 28  | 7.22 (3.79, 11.27) | 0.028*                  | 0.84 (0.47, 1.60)        |
| 40 ms | 90%    | 28  | 5.75 (2.09, 11.29) | 0.007*                  | 0.72 (0.20, 0.833)       |
|       | 110%   | 26  | 5.67 (1.83, 10.18) | 0.029*                  | 0.52 (0.18, 0.90)        |
|       | 130%   | 25  | 8.29 (4.66, 11.98) | 0.004*                  | 0.89 (0.49, 1.66)        |

* $p_{\text{corrected}} < 0.05$.

### Table 2

| ISI   | CS/rMT | $n$ | IHI (% 95% CI)/% | $p_{\text{corrected}}$ | Cohen’s $d$ (95% BCA CI) |
|-------|--------|-----|-----------------|-------------------------|-------------------------|
| 8 ms  | 90%    | 24  | 1.23 (0.56, 1.8) | 0.726                   | 0.61 (0.30, 0.70)        |
|       | 110%   | 23  | 0.33 (0.41, 2.2) | 0.726                   | 0.05 (0.36, 0.66)        |
|       | 130%   | 21  | 2.13 (0.1, 0.7)  | 0.726                   | 0.21 (0.20, 0.88)        |
| 10 ms | 90%    | 22  | 2.34 (0.25, 0.81) | 0.726                   | 0.25 (0.01, 0.26)        |
|       | 110%   | 24  | 0.68 (0.24, 0.41) | 0.726                   | 0.03 (0.18, 0.33)        |
|       | 130%   | 23  | 2.60 (0.56, 0.81) | 0.686                   | 0.25 (0.25, 0.59)        |
| 40 ms | 90%    | 23  | 2.02 (1.59, 5.3)  | 0.726                   | 0.31 (0.23, 1.06)        |
|       | 110%   | 22  | 5.46 (0.19, 10.8) | 0.368                   | 0.45 (0.02, 1.02)        |
|       | 130%   | 23  | 4.09 (0.37, 8.06) | 0.368                   | 0.37 (0.08, 0.69)        |

* $p_{\text{corrected}} < 0.05$.

### Table 3

| ISI   | CS/rMT | $n$ | IHI (% 95% CI)/% | $p_{\text{corrected}}$ | Cohen’s $d$ (95% BCA CI) |
|-------|--------|-----|-----------------|-------------------------|-------------------------|
| 10 ms | 110%   | 32  | 9.33 (5.48, 1.07) | <0.001*                 | 0.88 (0.53, 1.56)        |
|       | 120%   | 32  | 10.01 (6.30, 1.421) | <0.001*                 | 1.11 (0.61, 2.07)        |
|       | 130%   | 32  | 14.94 (10.47, 1.96) | <0.001*                 | 1.24 (0.66, 1.94)        |
| 40 ms | 110%   | 31  | 8.34 (5.31, 1.173) | <0.001*                 | 1.11 (0.57, 1.85)        |
|       | 120%   | 30  | 9.82 (5.05, 1.757) | <0.001*                 | 0.96 (0.50, 1.67)        |
|       | 130%   | 30  | 12.44 (8.43, 16.7) | <0.001*                 | 1.04 (0.05, 2.1)         |

* $p_{\text{corrected}} < 0.05$.

### Table 4

| ISI   | CS/rMT | $n$ | IHI (% 95% CI)/% | $p_{\text{corrected}}$ | Cohen’s $d$ (95% BCA CI) |
|-------|--------|-----|-----------------|-------------------------|-------------------------|
| 10 ms | 110%   | 25  | 6.69 (2.83, 10.6) | 0.007*                  | 0.75 (0.35, 1.40)        |
|       | 120%   | 22  | 8.58 (5.60, 11.2) | <0.001*                 | 1.05 (0.57, 1.74)        |
|       | 130%   | 27  | 10.26 (5.24, 11.49) | <0.001*                 | 0.79 (0.28, 1.84)        |
| 40 ms | 110%   | 26  | 5.73 (0.24, 9.29) | 0.029*                  | 0.59 (0.19, 1.31)        |
|       | 120%   | 25  | 8.37 (3.86, 13.5) | 0.010*                  | 0.78 (0.35, 1.53)        |
|       | 130%   | 23  | 9.02 (7.57, 12.79) | <0.001*                 | 1.17 (0.69, 2.17)        |

* $p_{\text{corrected}} < 0.05$.

IHI increased as a function of CS intensity. In particular, we found that the magnitude of M1-M1 IHI elicited with a CS intensity of 130% rMT was significantly greater than that evoked with CS intensities of 120% rMT ($t$ (17) = 3.26, $p_{\text{corrected}} = 0.009$, $d = 0.72$ (0.28, 1.60)) and 110% rMT ($t$ (18) = 3.32, $p_{\text{corrected}} = 0.009$, $d = 0.88$ (0.26, 1.49)). There was no significant difference between the levels...
of M1-M1 IHI associated with the 120% rMT and 110% rMT CS conditions ($p_{\text{corrected}} > 0.05$).

### 3.4. M1-M1 interhemispheric inhibition (AP test stimuli)

M1-M1 IHI with the test coil oriented to induce AP current flow was reliably expressed across all conditions (Table 4 and Fig. 3b). A robust rank-based non-parametric model with CS intensity (levels = 110%, 120%, 130% rMT) and ISI (levels = 10 ms, 40 ms) as fixed effects revealed a main effect of CS intensity ($F(1.83, \infty) = 3.91, p = 0.023$). There was no effect of ISI ($p = 0.496$) or a CS intensity × ISI interaction ($p = 0.959$). Post-hoc Hochberg-corrected Yuen’s t-tests did not however indicate the presence of reliable pairwise differences in M1-M1 IHI (elicited with AP test stimuli) between individual CS intensities (all $p_{\text{corrected}} > 0.05$).

### 3.5. Threshold hunting targets and control MEP amplitudes

The robust tests of equivalence indicated that the amplitudes of control MEPs elicited separately in each block (e.g. PMd-M1 IHI...
with PA test stimuli) varied about the expected THT amplitude of 200 µV (Table 5). This suggests that the TS intensity required to evoke a response equivalent to the THT amplitude (in the absence of a preceding CS) did not vary significantly over the course of the experiment. The THT was equivalent to 115% rMT in the PA condition (mean rMT\textsubscript{PA} = 43.3, sd = 9.44; mean THT\textsubscript{PA} = 49.7, sd = 11.44) and 113% rMT in the AP condition (mean rMT\textsubscript{AP} = 56.6, sd = 11.19; mean THT\textsubscript{AP} = 63.7, sd = 13.47). All means and standard deviations are expressed as percentages of maximum stimulator output.

4. Discussion

Here we established the effects of varying such parameters as CS intensity, ISI and test coil orientation on the magnitude of ATH-derived PMd-M1 IHI, assayed using a fully automated dual-coil TMS methodology. In doing so, we highlight for future application the parameter combinations with which PMd-M1 (and M1-M1) IHI may be most effectively assessed.
4.1. PMd-M1 IHI is dependent on test coil orientation and CS intensity

The specific outcomes of the present study indicate that the expression of PMd-M1 IHI is contingent on the test coil orientation. PMd-M1 IHI was present when the test coil was oriented to induce PA current flow, but not when it was oriented to induce current flow in the AP direction. This suggests that the expression of the phenomenon may rely upon functionally discrete populations of neurons. In the former condition, the magnitude of PMd-M1 IHI also increased as a function of CS intensity. This finding highlights the importance of carefully selecting the CS intensity should one wish to apply the parameter as a means of assaying interhemispheric motor cortical pathology. Specifically, in circumstances in which a low CS intensity is employed, a weak reference measure may be obtained, against which disease-related changes in IHI may be difficult to detect. In contrast, a CS intensity that is too large may give rise to ceiling effects. Indeed, in some instances the TS intensity that is required to maintain the target MEP amplitude may exceed the output capacity of the stimulator. There was no evidence that the level of PMd-M1 IHI was varied as a function of the ISI, which may indicate that PMd neurons activated at different ISIs may exert a relatively homogeneous influence upon neurons in the contralateral M1 (or conversely that the same sets of neurons are sampled at each ISI). A detailed discussion of the underlying physiological basis of this latter finding is however beyond the scope of the present study.

It has been demonstrated previously that by altering the orientation of the test coil, early (e.g. I1) or later I-waves (e.g. I2) may be elicited preferentially by cortical eddy currents that flow respectively in the PA or AP directions (Di Lazzaro et al., 2001, 2018; Di Lazzaro and Rothwell, 2014). The implication is that corticospinal volleys generated via different TMS coil orientations may reflect the contributions of somewhat distinct populations of neurons (Hamada et al., 2014; Hannah and Rothwell, 2017; Volz et al., 2014). It has been established that the application of conditioning stimuli to the opposite M1 primarily modulates the amplitudes of I1 waves (and, to a limited extent, I2 waves) generated by a TS applied subsequently to M1. There is not however any evidence of a corresponding effect on I1 waves (Di Lazzaro et al., 1999). On this basis it has been inferred that if the test coil is oriented to induce AP current flow, a more sensitive measure of M1-M1 IHI may be obtained (Mooney et al., 2018). In the present study, when the test coil was oriented to induce AP current flow, conditioning stimuli applied to the opposite PMd failed to generate reliable IHI. In contrast, when the TS was oriented to instead induce PA current flow, IHI was present for most ISIs (8, 10 and 40 ms) and CS intensities (90%, 110%, 130% rMT). This pattern of outcomes does not accord with the assumption that the amplitude of MEPs elicited by test stimuli that induce AP current flow in M1 are reflective of the state of long range corticocortical afferent fibres to a greater degree than test stimuli that induce PA currents. As noted above, although callosal fibres fit squarely the definition of long range corticocortical afferent fibres, it is thought that they ramify in multiple layers (Cissé et al., 2003). In future work therefore, consideration might be given to the possibility that in the context of IHI, it may not only be the origin of the afferent fibres (i.e. M1 versus PMd) that determines relative sensitivity to AP and PA currents, but also the distribution of the cortical layers to which the callosal fibres project.

4.2. M1-M1 IHI and the effect of CS intensity

When the CS was applied to M1, IHI was expressed when the TS induced either PA or AP current flow. This is consistent with previous findings (Ghosh et al., 2013; Mooney et al., 2018). The effect size estimates suggest that the depth of inhibition was somewhat larger when the TS induced PA, rather than AP, current flow. As with PMd-M1 IHI in the PA condition, and in accordance with extant reports (Ibey et al., 2015; Ni et al., 2008), the degree of M1-M1 IHI also increased as a function of CS intensity. This was not however the case when the TS induced AP current flow. Taken together with studies in which differential effects of AP and PA stimulation were observed with aging (Mooney et al., 2018), our findings suggest that in registering M1-M1 IHI, AP and PA test coil orientations capture physiologically distinct phenomena, and should therefore both be employed when assessing clinical populations. Irrespective of the test coil orientation, there was no effect of ISI on the magnitude of M1-M1 IHI that was registered (Uehara et al., 2014).

4.3. Limitations

A key limitation of the present study concerns the craniometric method by which the position of the PMd hotspot was determined, and the possibility of current spread (particularly at higher CS intensities, e.g. 130% rMT) to non-PMd areas. It has been suggested that the craniometric approximation of PMd that we and others (Mochizuki et al., 2004) have employed corresponds to a dorsolateral premotor site that lies at the border of the dorsal and ventral premotor cortices (PMv) (Fiori et al., 2017). While this position falls within the meta-analytically defined boundaries of PMd (Fiori et al., 2017), the possibility exists that the cortical eddy current induced by the CS at higher intensities may have spread to (and subsequently activated) cells in PMv. It has been demonstrated previously that conditioning PMv with peri-threshold CS intensities (90–110% rMT) at both short- (8 ms) and long-latency ISIs (40 ms) diminishes the amplitude of MEPs elicited from the contralateral M1 (Buch et al., 2010; Fiori et al., 2017). Consequently, we cannot exclude the possibility that the PMd-M1 IHI effect reported herein may be partially mediated by interhemispheric input from PMv.

Owing to the proximity of PMd to M1, there exists also a possibility that current spread from PMd to M1 may have contributed to the PMd-M1 IHI effect. We do not however consider this to be

| IHI Block   | Mean MEP/μV | SD MEP/μV | ys t | df  | p       |
|------------|-------------|-----------|------|-----|---------|
| PA PMd-M1  | 214.38      | 67.75     | 0.70 | 17  | <0.001* |
| AP PMd-M1  | 247.06      | 94.06     | 1.44 | 14  | <0.001* |
| PA M1-M1   | 242.14      | 77.92     | 1.81 | 18  | <0.001* |
| AP M1-M1   | 232.62      | 62.17     | 1.51 | 14  | <0.001* |

p < 0.05.
likely. In the first instance, it is well established that the application of subthreshold conditioning stimuli (e.g. 90% rMT) to M1 does not affect the amplitudes of MEPs elicited by test stimuli delivered subsequently to the opposite M1, at least at ISIs that conventionally engender IHI (De Gennaro et al., 2004; Ferbert et al., 1992; Hanajima et al., 2001; Mochizuki et al., 2004). Consequently, the effect of current spread from PMd to M1 at subthreshold conditioning stimulus intensities (i.e. 90% rMT) should be considered negligible (Mochizuki et al., 2004). Secondly, while the probability of current spread to M1 is certainly higher at suprathreshold CS intensities (i.e. 110% & 130% rMT), one would expect the level of PMd-M1 IHI assessed with both test coil orientations (AP, PA) to be similarly affected. It is notable therefore that while IHI was expressed in the majority of PMd-M1 PA conditions, it was conspicuously absent in all PMd-M1 AP conditions.

Finally, it should also be noted that while the electrophysiological phenomenon of IHI can be elicited in a relatively straightforward fashion using TMS techniques such as those used in the present study, it does not necessarily provide a faithful representation of the functional interhemispheric interactions that occur in normal, or indeed pathological, physiological conditions (Carson, 2020). In seeking to apply IHI as a quantitative biomarker therefore, considerable circumspection is necessarily required.

5. Conclusions

We have demonstrated that IHI between PMd and M1 is only obtained with test stimuli that induce current flow in the PA direction and that the magnitude of the effect increases as a function of CS intensity. In investigating PMd-M1 IHI in clinical populations, a PA test coil orientation, and CS intensities that provide a basis upon which to capture (pathological) elevation or diminution of this measure should therefore be employed. The absence of IHI when the CS was applied to PMd – in circumstances in which the TS induced AP current flow in the opposite M1, emphasises that the anatomical and physiological factors that determine the impact of test coil orientation on TMS-evoked potentials remain poorly understood.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to thank Prof. Friedemann Awiszus for his assistance in developing the ATH Signal-MATLAB interface. We also thank Micheala McIlvenna and Nicola Stead for their assistance with data collection.

Funding

The research reported herein was supported in part by the Irish Research Council [grant nos. GOIPG/2016/187, GOIPG/2017/1014].

Author contributions

GHMC, RM, and BGC conceived and designed the experiments. GHMC performed data acquisition. GHMC, RM, and RGC performed data analysis. GHMC, RM, and RGC wrote the paper. All authors approved the final article.
