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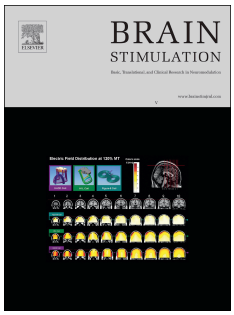

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TMS brain mapping in less than two minutes

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Abstract

Background: Transcranial magnetic stimulation (TMS) corticospinal excitability maps are a valuable tool to study plasticity in the corticospinal tract. Traditionally, data acquisition for a single map is time consuming, limiting the method's applicability when excitability changes quickly, such as during motor learning, and in clinical investigations where assessment time is a limiting factor.

Objective: To reduce the time needed to create a reliable map by 1) investigating the minimum interstimulus interval (ISI) at which stimuli may be delivered, and 2) investigating the minimum number of stimuli required to create a map.

Method: Frameless sterotaxy was used to monitor coil position as the coil was moved pseudorandomly within a 6 x 6 cm square. Maps were acquired using 1-4 s ISIs in 12 participants. The minimum number of stimuli was determined by randomly extracting data and comparing the resulting map to the original data set. To confirm validity, the pseudorandom walk method was compared against a traditional mapping method.

Results: Reliable maps could be created with 63 stimuli recorded with a 1 s ISI. Maps created acquiring data using the pseudorandom walk method were not significantly different from maps acquired following the traditional method.

Conclusions: To account for inter-participant variability, outliers, coil positioning errors and, most importantly, participant comfort during data acquisition, we recommend creating a map with 80 stimuli and a 1.5 s ISI. This makes it possible to acquire TMS maps in two minutes, making mapping a more feasible tool to study short- and long-term changes in cortical organisation.

Introduction

For nearly 30 years, transcranial magnetic stimulation (TMS) has been a valuable tool to study plasticity of the human primary motor cortex (M1), with the first TMS maps being documented in the early 1990s [e.g. 1, 2]. Initially, the technique was time consuming and imprecise; however, the development of navigated brain stimulation using frameless stereoscopy [3] improved its repeatability [4, 5]. Despite this step forward, the mapping method remains a time consuming technique and its use beyond the research environment remains limited to pre-surgical tumour mapping [6]. The importance of reducing acquisition time is evident from the observation that corticospinal excitability fluctuates with time [7, 8] and attention [9, 10], and any changes following motor learning are short lasting. Moreover, in clinical practice the time available with a patient is limited. Lengthy TMS protocols are both mentally and physically demanding for the patient, thus limiting their use. As a result, numerous studies have reduced acquisition time by compromising the map quality.

Traditionally, data acquisition for a full map requires between 15-30 min [11-13], and this can take up to 1 hour dependent on the protocol employed [14]. Importantly, this acquisition time does not include preparation time to set up the electromyographic (EMG) recording, determine the most excitable scalp site (commonly referred to as the hotspot) or to determine motor thresholds. Data is typically acquired by stimulating M1 at multiple predefined sites, organised in ~1 cm spaced rows and columns (See Figure 1A), with 3-5 stimuli delivered at each site [e.g. 2, 15]. Offline, the position data are then matched to motor evoked potentials (MEP) acquired from the EMG data to produce a 2-dimensional contour plot (see Figure 1C). To reduce acquisition time many investigators now use some combination of shorter interstimulus interval, fewer stimulation sites or fewer stimuli per site.

In the literature, as few as 11 and as many as 225 stimulation sites have been reported [16, 17]. Sites are usually distributed in a square or rectangular grid with spaced at 1–2 cm [e.g. 18]. Between 3–10 stimuli are typically administered per site [2, 15, 19-21] and the ISI is typically set between 3–6 s, although reports in the literature range from 1.1–15 s [15, 18, 22-24]. Acquisition time has been reduced to as little as 2.5–10 min [e.g. 23, 24, 25],

although this is achieved by minimising the number of stimulation sites [e.g. 25] or reducing the ISI [e.g. 23, 24]. However, the effect on the TMS map has not been validated against the more traditional long mapping protocols. This observation is interesting, as compromises with any of the mapping acquisition parameters has been observed to shift the centre of gravity (COG) of the map, and to change its area and/or volume, with respect to the 'true' values [26, 27]. This highlights the importance of parameter selection. There is, however, no consensus in the literature about how best to optimise these parameters in order to produce a good-quality map in a short period of time.

Grey et al. [28] used frameless stereotaxy and a pseudorandom walk approach to avoid the problem of accurate coil positioning to predefined targets (see Figure 1A). When delivering single stimuli in a pseudorandom walk one does not need to repeatedly place the coil in a specific predefined position and orientation, thus ISI may be decreased in order to shorten the acquisition time. No statistically significant difference was observed comparing the grid system (traditional method) and random walk method for either of the COG x-y coordinates, suggesting the two methods are comparable. More recently Julkunen [29] confirmed that it is not necessary to use an evenly spaced stimulus grid in order to create a reliable map.

By adopting a pseudorandom walk method the stimulation site spacing and number of stimuli per site become redundant parameters. As a result it is only necessary to consider the ISI and the number of stimuli. The aim of this study was to use the pseudorandom walk method to minimise the duration of the data acquisition (excluding preparation and data analysis) required to construct a TMS map. This minimises the effect of changing attention on corticospinal excitability and allows the method to be more feasible for motor learning and clinical assessments. Therefore, we first determined the minimum ISI at which stimuli could be delivered. Specifically, we examined five ISIs (1, 1.5, 2, 3 and 4 s) and tested the hypothesis that ISIs of 1, 1.5, 2 and 3 s would be different from 4 s [11, 13, 18, 30-32], as evidenced by changes in COG, map area and map volume. Second, we determined the minimum number of stimuli needed to create a map, therefore combining the minimum ISI and minimum number of stimuli in order to determine the time needed to create a map.

Finally, to ensure validity of the method, we compared maps generated with the pseudorandom walk method to maps generated with the traditional method of data acquisition. This was achieved by comparing COG, map area and map volume and assessing comparing reliability of both methods.

Methods

Participants

In total, 12 healthy participants were recruited for both experiments in this study (Experiment 1: 24.2 ± 7 y, range 20-46, 5 female; Experiment 2: 23.2 ± 6 y, range 18-35, 8 female), with some participating in both experiments. Participants were screened for contraindications to TMS using a modified version of the TMS adult safety questionnaire [33]. The study was approved by the University of Birmingham's Science, Technology, Engineering and Mathematics ethics committee (ERN_12-1189), and all experiments were performed in accordance with the Declaration of Helsinki.

Electromyography

Bipolar surface electrodes (Blue Sensor N, Ambu, Denmark) were used to record the electromyographic (EMG) activity of the first dorsal interosseus (FDI). All EMG signals were amplified (500-2k), band pass filtered (20-1000 Hz), and digitally sampled at 5 kHz to be stored for offline analysis.

Transcranial Magnetic Stimulation

Magnetic stimulation was delivered with a Magstim Rapid^2 (Magstim Ltd, Dyfed, United Kingdom), using a custom made polyurethane coated 90 mm figure-of-8 coil. The coil was held at 45 deg to the sagittal plane with the handle pointing in posterior direction to induce biphasic currents in the lateral-posterior to medial-anterior direction, optimal for exciting the area associated with hand and arm muscles [26, 34]. Stimuli were delivered at a constant participant-specific intensity until the coil position on the scalp that evoked the largest MEP was found (commonly referred to as the hotspot). The hotspot was then marked as a target with the neuronavigation system. With the coil on the hotspot, the resting motor threshold (RMT) was determined according to the definition of Rossini [35, 36], as the threshold at which 5 out of 10 stimuli evoked an MEP with a peak-to-peak amplitude of 50 µV. In a very few number of cases, this definition could not be used due to noise in the electromyogram

that just exceeded 50 μV. In these cases the threshold was determined as the intensity at which at least 5 out of 10 stimuli evoked an MEP clearly discernible from background EMG. Coil position and orientation were monitored throughout the experiment using frameless stereotaxy (BrainSight 2, Rogue Research Inc, Montreal, Canada). To create a map, stimuli were delivered within a rectangular 6 x 6 cm grid superimposed on a generic brain image in the Brainsight 2 software (see Figure 1A). The grid was placed relative to surface anatomy landmarks (e.g. vertex and ears) in an area that would encompass the hand area of the motor cortex.

Peripheral Nerve Stimulation (PNS)

MEPs were normalised to the electrically evoked maximal M-wave (M_{max}) in order to compare across different participants. To obtain the M_{max}, a bipolar probe was used to stimulate the medial nerve at the level of the elbow using a constant current stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Garden City, UK).

Experimental protocol

The participants were seated comfortably in a chair with the right hand resting pronated on a table. Participants were instructed to keep the hand fully relaxed during the experiments.
The participants were seated comfortably in a chair with the right hand resting pronated on a table. Participants were instructed to keep the hand fully relaxed during the experiments.
Online feedback of FDI EMG was provided by displaying a colour, green or red, based on the participant's root mean square EMG to ensure compliance with this instruction and to focus attention. No direct feedback of the raw EMG was provided to either the experimenter or the participant. One expert TMS experimenter performed all of the testing.

Experiment 1: Effect of Interstimulus Interval (ISI) and Minimum Number of Stimuli (N_{stim})

To improve the temporal resolution, this experiment was designed to investigate the effect of ISI and the number of stimuli on centre of gravity (COG), map area and map volume. This

experiment was performed with 12 participants. The effect of stimulation frequency was studied using five different ISIs: 1, 1.5, 2, 3 and 4 s. A maximum ISI of 4 s was chosen because an ISI of 3-6 s is commonly reported [11, 13, 18, 30-32] and to ensure the experiment would not last longer than 2 hours. Each map was created by applying 100 stimuli at 120% RMT in the predefined grid. Stimuli were delivered to random locations within the 6 x 6 cm square. The objective was to ensure two successive stimuli were not delivered in close proximity and that that final map was populated by stimuli with a roughly equal spread across the grid (Figure 1A). Immediate feedback about stimuli position and orientation were provided by position markers in the neuronavigation display. Three maps were collected for each ISI, with the order of presentation randomised to avoid an ordering effect. To ensure participants would remain focussed on their task, a rest period of 1-2 min was given between the maps.

Experiment 2: Validation to traditional mapping protocol

This experiment, performed with 12 participants, was designed to validate if a map created using the characteristics found in Experiment 1 would compare to a map using the traditional method. For the traditional method a 6 × 6 cm grid was created from 7 rows and 7 columns with 1 cm spacing. Three stimuli were administered to each site at 120% RMT using a 1.5 s ISI. Maps acquired using the traditional method were compared to maps acquired using the pseudorandom walk method with 80 stimuli at 120% RMT and a 1.5 s ISI as determined in Experiment 1 (See Results Experiment 1). Three maps were collected for each method, with order of presentation randomised to avoid an ordering effect. Similar to Experiment 1, a 1-2 min rest period was provided between maps.

Data analysis

Figure 1 illustrates how the EMG and neuronavigation data were combined to construct a TMS map. Maps were created offline with a bespoke MATLAB script **(**MATLAB Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States). First, the MEP was

quantified by the peak-to-peak value (MEP_{pp}) extracted from a window 20—50 ms after stimulation (Figure 1B). The corresponding stimulation position was extracted from the neuronavigation data and transposed into a 2D plane. An approximant based surface modelling tool [37], was used to fit a surface through the transposed data. An example of a map in both 3D and 2D are shown in Figure 1C. A more detailed description of the data processing may be found in the supplementary material. Individual stimuli within a map were excluded from analysis if the stimulation or corresponding MEP did not fulfil one of four conditions: 1) the root mean square value of the background EMG (50 - 5 ms before stimulation) was within Mean ± 2 *SD* of all stimuli; 2) stimulation at most 10 mm outside the grid border; 3) MEP size not larger than Mean ± 3.5 *SD* of all MEPs in the map; 4) angle and translation of stimulus within 99% predication interval of all stimuli.

Figure 1 approximately here

Statistical Analysis

Statistical testing was conducted with NCSS 2007 v07.1.4. Tests were considered significant at $\alpha = 0.05$. As the descriptive statistics showed much of the data violated the standard assumptions of normality (typical positively skewed or uniformly distributed) and equal variance, non-parametric statistics were used for the analysis.

Experiment 1: Effect of Interstimulus Interval (ISI)

COG was compared between ISIs using the Euclidean distance, hereafter referred to as distance, between each COG and the average COG of ISI = 4 s. An ISI of 4 s was chosen as the benchmark as an ISI between 3-6 s is most commonly used [11, 13, 18, 30-32]. COG, area and volume were tested using the non-parametric Friedman Test across ISI. Planned post hoc comparisons were performed using the Wilcoxon Signed-Rank Test between ISI = 4 s and all other ISIs. A Bonferroni adjustment was applied to compensate for the multiple comparisons; therefore, in this case $\alpha = 0.0125$ was used for significance.

223

224 *Minimum Number of Stimuli*

225 Post processing to obtain the minimum number of stimuli (N_{stim}) was required to produce a
226 reproducible map. Stimuli were randomly extracted from the map, the map was
227 reconstructed and the correlation coefficient (r^2) was calculated to compare the original and
228 reconstructed map. A map was considered significantly different if either the COG distance
229 exceeded 3.6 mm (75th percentile of COG variability – See Results – Experiment 1) or the r^2
230 parameter dropped below 0.9.

231

232 *Experiment 2: Validation to traditional mapping protocol*

233 Mean COG of both the traditional and random mapping method was compared using the
234 Wilcoxon Signed-Rank Test. Area and volume were compared using the non-parametric
235 Friedman Test. Post-hoc comparisons were assessed using the Wilcoxon Signed-Rank
236 Test. We also examined the reliability of the parameters of the map for both the traditional
237 and the random walk method using the intraclass correlation coefficient (ICC). Measurement
238 reliability was defined according to the ICC, with ICC ≥ 0.75 defined as excellent reliability,
239 ICC between 0.50 - 0.74 as moderate reliability, and ICC ≤ 0.49 as poor reliability [38, 39].
240 The pseudorandom walk method was considered valid when no significant differences for
241 the parameters between the methods were found or, if differences were found, they fell
242 within observed variability. Moreover, the reliability of the COG and map area had to be
243 moderate to excellent (ICC ≥ 0.50). Map volume was not considered in this assessment as
244 findings with respect to reliability are inconclusive [13, 21, 23, 32]. In addition, to classify the
245 between and within-subject variance the quartile coefficient of dispersion (QCD) and
246 standard error of measurement (SEM) was calculated [40]. SEM was calculated for all map
247 parameters as the square root of the mean square error (MSE): $SEM = \sqrt{MSE}$. The QCD
248 was calculated for map area and volume using: $QCD = \frac{Q_{75}-Q_{25}}{Q_{75}+Q_{25}}$, where Q_{25} and Q_{75} are the
249 25th and 75th percentile. The centre of gravity measures were excluded from the between

250 subject analysis because we used a generic structural scan for participants. A between
251 participant analysis of centre of gravity was therefore not valid.

Results

Data exclusion

All participants tolerated the TMS well and completed the study. Individual stimuli were excluded based on background EMG, coil angle and translation, position relative to the grid and MEP size. In total 8.2% of all stimuli were excluded before analysing the maps (180 maps analysed). Most stimuli were excluded due to either high background EMG (4.2% of the total number of stimuli) or angle and translation of the stimulus with respect to the skull (3.3% of the total number of stimuli). On average, 8.5 (IQR: 7 ± 11) stimuli were excluded per map.

Experiment 1: Effect of Interstimulus Interval (ISI)

In order to study the effect of ISI on the TMS map we compared five different ISIs (1, 1.5, 2, 3 and 4 s). TMS maps collected with 1, 2 and 4 s ISI from a representative participant are shown in Figure 2.

Figure 2 approximately here

The maps with stimuli delivered at 1 s and 2 s are very similar in shape and activity compared with the 4 s ISI map. In addition, COG is similar in all three maps across all participants, although the Freidman's test used with the group data revealed a small, but significant difference for COG between the four ISIs ($\chi^2(4) = 17.87$, $P < 0.01$). Post hoc comparisons revealed small differences between ISIs of 1.5, 2 and 3 s compared with 4 s, for the Bonferroni adjusted P-value (0.0125), whilst there was no significant difference between ISIs 1 s and 4 s ($Z = 1.56$, $P = 0.12$, Figure 3A). The COGs of 4 s ISI differed less than 0.7 mm from all other ISIs. Overall, the median Euclidean distance between ISI 1, 1.5, 2 and 3 s compared with 4 s was 2.4 mm (IQR: 1.2 – 3.6 mm and 10/90th percentiles: 0.7 – 4.8 mm), with x-direction 1.3 mm (IQR: 0.6 – 2.3 mm) and in y-direction 1.1 mm (IQR: 0.5 – 2.5

mm). Neither map area nor map volume revealed significant differences with ISI (area: $\chi^2(4) = 0.47$, P = 0.98; volume: $\chi^2(4) = 1.07$, P = 0.90) (Figure 3B|C).

Figure 3 approximately here

Minimum number

All 180 data sets were analysed in order to calculate the minimum number required to produce a map. In all cases the maps with reduced stimuli were well correlated with the original map with the full complement of data until very close to the minimum cut-off, as determined by a drop in r^2 or a shift in COG. In 95% of the cases, the minimum number was determined by r^2 crossing the 0.9 threshold rather than the COG shifting more than 3.6 mm. Figure 4A is a representative example of a set of maps calculated from the same data set.

Figure 4 approximately here

In this case 6 stimuli were excluded because the background EMG exceeded the activation cut-off, leaving 94 stimuli for the full map. The correlation coefficient dropped below 0.9 after 38 stimuli were randomly removed from the analysis, leaving a minimum number for this data set of 56 stimuli. A map from this data set with 24 stimuli ($r^2 = 0.78$) and a different contour is also illustrated. The decrease of r^2 by extracting stimuli from the map is illustrated in Figure 4B, dropping below 0.9 at 56 stimuli. Figure 5 shows the minimum number of stimuli calculated across 15 maps for each participant, sorted from participants with the highest to lowest average number of stimuli. This figure highlights the considerable spread in minimum number of stimuli needed to create a map. The median minimum number of stimuli was calculated across all participants as 63 (IQR: 46-74).

Figure 5 approximately here

Experiment 2: Validation to traditional mapping protocol

To validate the pseudorandom technique, a control experiment was conducted to determine if maps collected with this method were comparable to maps acquired in the traditional manner. TMS maps with the two different methods from a representative participant are shown in Figure 6A. The stimulation sites are marked with black open circles.

Figure 6 approximately here

It can be observed that the map created using the pseudorandom method is very similar to the map created with the traditional method. No clear difference can be observed in COG and map area of the two methods. Two data sets were omitted from the analysis due excessive ambient noise in EMG recordings; therefore the analysis was performed on 10 participants. The boxplots for COG for both x and y directions are shown in Figure 6B. COG was significantly different between methods in Y (yCOG: $Z = 2.48$, $P = 0.01$) but not in X (xCOG: $Z = 1.89$, $P = 0.06$). However, the median xCOG and yCOG differed by only 1.2 mm and 2.1 mm, respectively, which falls within the IQR for COG variability observed in Experiment 1. Neither map area nor map volume was significantly different between methods (area: $\chi^2(1) = 0.40$, $P = 0.53$; volume $\chi^2(1) = 0.16$, $P = 0.21$).

ICCs, SEMs and QCDs for both the traditional and random walk are listed in Table I. ICCs for xCOG, yCOG and area were moderate to excellent (ICC > 0.74). However, the ICC of the volume for the random walk method was poor (ICC = -0.63). Whist small differences in SEM for xCOG and yCOG are observed, 0.7 mm and 0.3 mm, respectively, they are within the variance reported for xCOG and yCOG in Experiment 1. For map area the SEM was 343 for the traditional method and 323 for the pseudorandom method. This difference can be considered negligible with respect to its order of magnitude. For both map area and volume, QCD was smaller for the pseudorandom method (0.2) than the traditional method (0.3 - 0.4).

Table 1 approximately here

Discussion

We have demonstrated that it is possible to acquire a TMS map in less than two minutes by reducing the interstimulus interval and by taking advantage of frameless stereotaxy to deliver stimuli in a pseudorandom walk. In addition, we estimated the minimum number of stimuli required to create a TMS map was 63 (IQR: 46-74). To account for inter-participant variability in minimum number of stimuli, and stimuli excluded during data analysis (on average 7-11), we recommend using 80 stimuli. Maps created with the new method are very similar to maps created with the traditional mapping method where stimulation sites are predefined. Whilst maps can be created by acquiring data with an interstimulus interval up to 1 s, we recommend using at most 1.5 s to limit participant discomfort. As a result, maps constructed from 80 stimuli acquired with an ISI of 1.5 s can effectively reduce the acquisition time to two minutes.

How quickly can data be acquired for a TMS map?

The primary aim of the present study was to improve the acquisition time of the mapping method without reducing the quality of the map. The present study indicates the TMS map can be recorded with an ISI of 1s. Whilst significant differences in COG were observed between 1.5, 2, 3 and 4 s, they were always very small (< 0.7 mm), falling within the overall COG variability of 2.4 mm (IQR: 1.2 – 3.6 mm). The significant differences reported in this study can therefore be attributed to natural variability as caused by fluctuating corticospinal excitability. Most importantly, there was no difference in COG between maps acquired with ISIs of 1 s and 4 s. The 2.4 mm COG variability corresponds well to the 3 mm variability in COG reported by others using the traditional mapping method both within and between sessions [25, 27, 29, 41, 42] . The present study concentrated on within-session variability. We did not, however, examine between-session variability which has been shown to be larger (6 – 10 mm) [32, 43]. As a result, further testing is warranted to confirm the between session variability of the COG using the pseudorandom walk method.

The observation that the map does not change with shorter ISIs is not surprising. Whilst the use of a 1 s ISI has been associated with lasting depression of excitability of the cortex when administered to a single site repetitively for 4 - 15 min [44, 45], a number of recent observations suggest depression is unlikely to be a problem with the present method. For example, we have recently demonstrated that TMS delivered with an ISI of 1 s for 3 min to the same stimulation site does not change corticospinal excitability [46]. In addition, the use of the random walk method ensures the same site is not repeatedly stimulated and the possibility of reduced synaptic efficiency is further reduced. However, whilst we have demonstrated in the present study that the use of 1 s ISI is technically feasible, stimulating this quickly does have some drawbacks. For example, we have observed that inexperienced users find it difficult to move the coil to a new location with only 1 s ISI. In some cases this leads to increased experimenter error. We noticed some users were not able to maintain the coil orientation correctly on the scalp at the new location because they were focusing on the neuronavigation software rather than the participant's head. More importantly, some participants reported discomfort and anxiety when the stimuli where delivered with an ISI of 1 s and had difficulty complying with the instruction to relax the target muscle. For these reasons we advocate using an ISI of at least 1.5 s when mapping with this method, however emphasize that a 1 s ISI does not affect the TMS map if an experienced TMS user performs the mapping and the participant is comfortable with the procedure.

On average the minimum number of stimuli needed to create a reproducible map was 63 (IQR: 46-74). A considerable spread in the minimum number was found between participants (Figure 5), highlighting the importance of acquiring sufficient data for the TMS map in order to overcome this variability. In post-processing, 7-11 stimuli were excluded from analysis. Therefore, to ensure sufficient data is collected to produce a reproducible map we suggest a minimum of 80 stimuli are required for to produce a map with this method. Using an ISI of 1.5 s, a map can therefore be acquired in 2 min. It should be emphasized that this does not include setting up the EMG recording, co-registering the participant's head to the MRI, finding the hotspot and RMT, and processing of the data to create the map.

Map variability

The within session variability of the map parameters can mainly be attributed to MEP variability, although it has been confirmed that maps can be reliably created despite this variability [47]. MEPs are affected by attention [8-10], asynchronous firing of motor units with phase cancellation [48] and a variety of nonphysiological factors such as coil position and coil orientation [49-51]. In this study, we used the commonly adopted 45 degree coil angle to stimulate the motor cortex which is commonly believed to optimally excite the hand area [52]. Interestingly, it has been suggested that the optimal coil angle should be individually determined [53, 54]. However, the benefit is likely to be minor [4]. Whilst individualising the coil orientation might decrease MEP variability it would also increase the mapping time, which is not beneficial for clinical application. In addition the use of electrical field estimates as opposed to RMT has been advocated as a more reliable measure [51, 55], however this is not common practice. MEP variability also depends on the muscle studied and the stimulation site, with proximal muscles usually reported to have more variable MEPs than distal muscles. and variability increasing as the coil is moved away from the hotspot[26]. Map reliability has also been argued to be sensitive to experimenter error [32, 56]. In an attempt to reduce these sources of variability and improve the quality of the map we took several precautions both during data acquisition and in post-processing.

First, to ensure attention was maintained during data acquisition, participants were provided with continuous feedback about the level of EMG which they were instructed to keep between predefined boundaries. In general, participants reported this task as being easy to achieve but also that it required continuous focus to successfully perform. Whereas this task minimized and stabilised background EMG, any trials with increased background EMG were exclud-ed to further minimize MEP variability. Second, the neuronavigation data was scrutinised offline to ensure coil orientation was consistent throughout the session. Furthermore, the TMS map was made less sensitive to MEP variability by smoothing the data with a Matlab surface fitting tool called ‘gridfit’ [37]. Full details are available in the Supplementary Material. Briefly, local variability in the surface fit was filtered by setting the

compliance of the fit with a stiffness setting in the gridfit tool. This setting was determined through extensive pilot testing and maintained constant for all maps analysed in this study. This filtering is especially beneficial in the periphery of the map, where variability in the smaller MEPs has been argued to be source of reduced reliability of the map parameters [21]. As a result, the quality of the map is improved and the number of stimuli needed to construct a map is reduced without compromising information content.

For both the pseudorandom as the traditional method we found the greatest ICCs for xCOG and yCOG. In general most literature supports the notion that COG is a more reliable parameter than either area or volume [13, 21, 23, 32]. We confirmed for the pseudrandom walk method that also area is a reliable measure but this does not hold for volume. The difference in reliability of the map volume between the methods is in line with the equivocal reports earlier [13, 21] and is unlikely to be a consequence of the method. Therefore, we recommend focusing on COG and area when analysing TMS maps.

Further considerations

It is interesting to note the increased use of TMS mapping in neurosurgery as a tool for brain tumour localisation. This contrasts to its use in studying motor system plasticity and motor rehabilitation, where the technique remains confined to research studies. The present study indicates it may be possible to use a shorter ISI for presurgical mapping, where a 4 s ISI is common practise [6]. However, it must be emphasised that further study in this area is warranted and that the computational method should be validated against existing methods to determine corticomotor representation size [29].

The method to create a TMS map presented here makes it possible to assess cortical organisation in less than 2 minutes. We recommend using at least 80 stimuli to take account for variability. Whilst it is possible to use fewer stimuli an ISI of 1 s to produce a map in as little as 1 min, maps produced in this manner will be subject to greater error. To tackle the observed variability in the minimum number of data required to produce a map, a potential next step is to develop a system whereby maps are generated online as the data are

444 acquired to provide the researcher direct feedback about the map. Such a method could, for
445 example, use a parameter estimation algorithm (PEST) as has recently been used in this
446 field for threshold tracking [57]. This would negate the need for a minimum number of stimuli
447 as data could be acquired until a robust map is achieved. This would also give the
448 opportunity to improve spatial resolution in areas of interest such as the area in the
449 immediate proximity of the hotspot.

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459 **Figure captions**

460

461 **Figure 1:** A step-by-step illustration outlining the creation of a TMS map.
462 (A) The traditional mapping method is illustrated on the left and the pseudorandom walk
463 method on the right. The traditional mapping method makes use of a predefined, usually 1-
464 cm spaced grid of target locations, as indicated by the blue markers. Multiple stimuli are
465 successively delivered to each site. In contrast, the new method uses four blue markers to
466 define a boundary without specific targets and within which stimuli are delivered
467 pseudorandomly. The white arrows indicate the direction in which stimuli were acquired. For
468 clarity, these maps are as data are acquired rather than at the end of a trial. (B) A 6 x 6 cm
469 square grid is defined in the neuronavigation software (BrainSight 2.0, Rogue Research) and
470 each stimulation site is matched with the recorded EMG. The motor evoked potential's peak-
471 to-peak (MEP_{pp}) value is extracted in a window between 20-50 ms after stimulation. (C)
472 Using a bespoke MATLAB script, a surface is fitted through the 3D position data cloud to
473 create a 2D plane. The 2D position data are then matched with the MEP_{pp} data to fit a
474 surface map. This map can be viewed in either a 3D (left) or 2D (right) map. The colour bar
475 represents the MEP_{pp} normalised by the maximally evoked electrical response (M_{max}).

476

477 **Figure 2:** Single participant data illustrating TMS maps acquired at three interstimulus
478 intervals (1, 2, and 4 s) using a 6 x 6 cm grid and 100 stimuli at 120% of resting motor
479 threshold. Very similar maps were also acquired at 1.5 and 3 s, but are not shown in the
480 figure to aid clarity. Each black open circle represents the location of a stimulus.
481 Corticospinal excitability is indicated by colour, with blue representing lack of excitability and
482 red representing the greatest excitability. The black cross (X) highlights the centre of gravity.
483 In this participant, neither the centre of gravity, area or volume changed across the five ISIs.

484 **Figure 3:** Group data for the effect of interstimulus interval on TMS maps (n = 12). All box
485 plots show the median (black line in the box), interquartile range (IQR; box top and bottom)

and 10th and 90th percentiles (error bars). Five different ISIs (1, 1.5, 2, 3 and 4 s) were compared and three maps were acquired for every ISI. All statistical testing was performed using the non-parametric Friedman test. (A) Group data of the Euclidean distance of each interstimulus interval relative to the mean centre of gravity of an interstimulus interval of 4 s. Centre of gravity was found not to be different when maps where acquired with 1 s interstimulus interval compared to 4 s. Moreover, no difference was found for (B) map area and (C) map volume between interstimulus intervals ($P > 0.05$).

Figure 4: Single participant data illustrating the effect of reducing the number of stimuli on the TMS map. Minimum number of stimuli was determined by randomly extracting stimuli starting at 100 stimuli minus the stimuli removed based on criteria of background EMG, coil position and coil orientation (6 in this particular example). Stimuli were extracted at random one by one, calculating the correlation coefficient and change of centre of gravity with respect to the map containing all data. The minimum number was taken when the correlation dropped below 0.9 or the centre of gravity moved more than 3.6 mm (Euclidean distance). In this example the minimum number was taken at 56 when the correlation was 0.9. Removing more stimuli changes the map as shown when only 24 stimuli are left, while the correlation coefficient is still high (0.78). (A) The TMS maps with 94, 56 and 24 stimuli. (B) The correlation coefficient (r^2) plotted against the number of stimuli used to create the map. With 56 stimuli, r^2 dropped below 0.9.

Figure 5: The minimum number of stimuli for each participant (n=12), as determined from 15 maps that were collected in every participant. The participants have been sorted from a high to low average minimum number. All box plots show the median (black line in the box), interquartile range (IQR; box top and bottom) and 10th and 90th percentiles (error bars). The overall median (Mdn) of 63 stimuli and interquartile range (46-74) are presented by the solid and dashed horizontal lines. The minimum number was defined as when the map's correlation with respect to a map containing all data dropped below 0.9 or the centre of gravity moved by more than 3.6 mm (Euclidean distance).

Figure 6: Single participant data illustrating TMS maps acquired using the traditional method and the here proposed pseudorandom walk method. (A) For the traditional method mapping was acquired from 49 stimulation sites organised in 1-cm spaced rows and columns, each stimulated three times with an interstimulus interval of 1.5 s and at 120% of resting motor threshold. For the random method 80 stimuli were applied at random positions across the grid with an ISI of 1.5 s at 120% RMT. (B) Box plots for the group data of the x- and y-coordinate of the centre of gravity (xCOG and yCOG) for both the pseudorandom (shaded bars) and traditional method (white bars). Shown are the median (black line in the box), interquartile range (IQR; box top and bottom) and 10th and 90th percentiles (error bars). No differences were found for the xCOG, map area or map volume. However the yCOG was found to be significant between methods. Median difference for yCOG is 2.1 mm well within observed COG variability, therefore this significant change is not considered as a result of the method but rather map variability.

526 **Table caption**

527

528 **Table 1:** Intraclass correlation coefficients (ICCs), standard error of measurement (SEM)
529 and quartile coefficient of dispersion (QCD) for both the traditional and pseudorandom walk
530 mapping method, showing the test-retest reliability and variance of the mapping parameters.
531 Apart for volume, correlation is good to excellent for both methods. This indicates the
532 random walk method is a reliable method for creating TMS maps. The small differences in
533 SEM for both x- and y-coordinate of the centre of gravity (xCOG and yCOG) fall within 1.3
534 mm and 1.1 mm COG variances reported in Experiment 1. The SEM difference of 20 for
535 map area can be considered negligible with respect to its order of magnitude. QCD is
536 smaller for both map area and volume for the pseudorandom method compared to the
537 traditional method.

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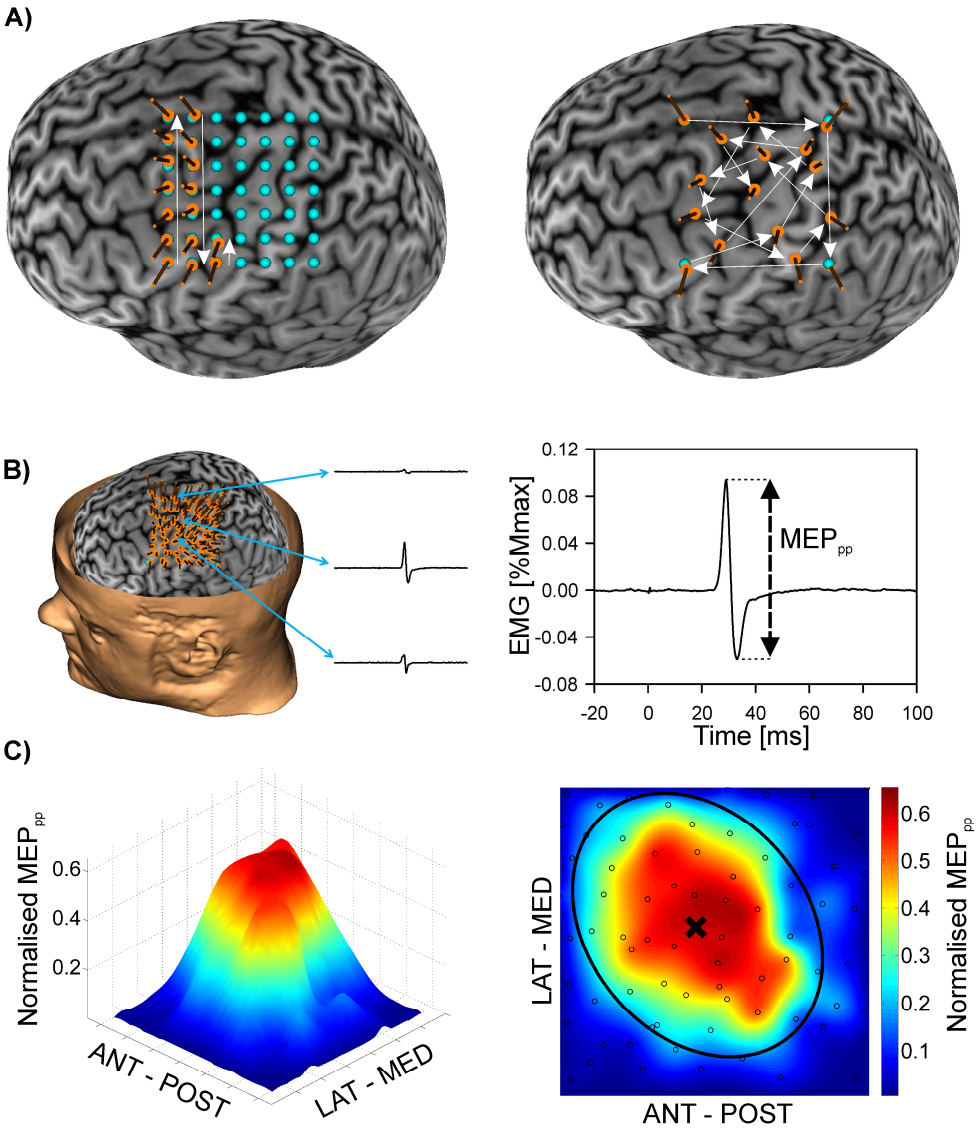
	Method					
	Traditional			Pseudorandom		
	ICC	SEM	QCD	ICC	SEM	QCD
xCOG	0.94	1.63	x	0.82	2.30	x
yCOG	0.92	1.62	x	0.92	1.93	x
Area	0.87	343.39	0.32	0.74	323.41	0.21
Volume	0.76	0.14	0.44	-0.63	0.20	0.22

Table 1: Intraclass correlation coefficients (ICCs), standard error of measurement (SEM) and quartile coefficient of dispersion (QCD) for both the traditional and pseudorandom walk mapping method, showing the test-retest reliability and variance of the mapping parameters. Apart for volume, correlation is good to excellent for both methods. This indicates the random walk method is a reliable method for creating TMS maps. The small differences in SEM for both x- and y-coordinate of the centre of gravity (xCOG and yCOG) fall within 1.3 mm and 1.1 mm COG variances reported in Experiment 1. The SEM difference of 20 for map area can be considered negligible with respect to its order of magnitude. QCD is smaller for both map area and volume for the pseudorandom method compared to the traditional method.


A)
B)
EMG [%Mmax]
0.12
0.08
0.04
0.00
-0.04
-0.08
MEP$_{pp}$
-20
0
20
40
60
80
100
Time [ms]
C)
Normalised MEP$_{pp}$
0.6
0.4
0.2
ANT - POST
LAT - MED
LAT - MED
ANT - POST
0.6
0.5
0.4
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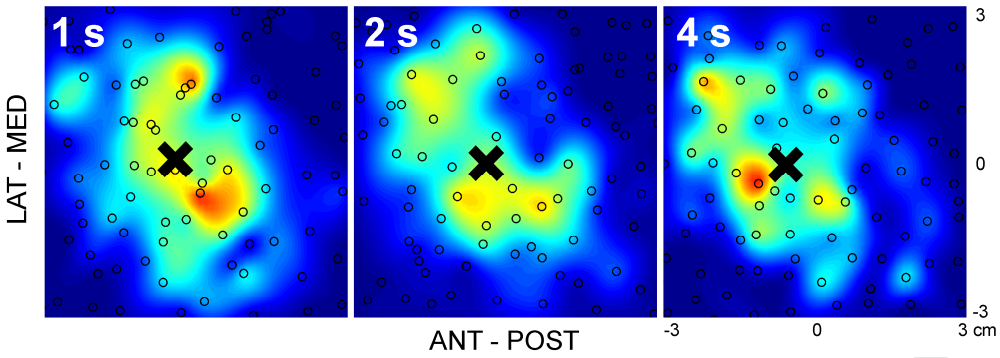
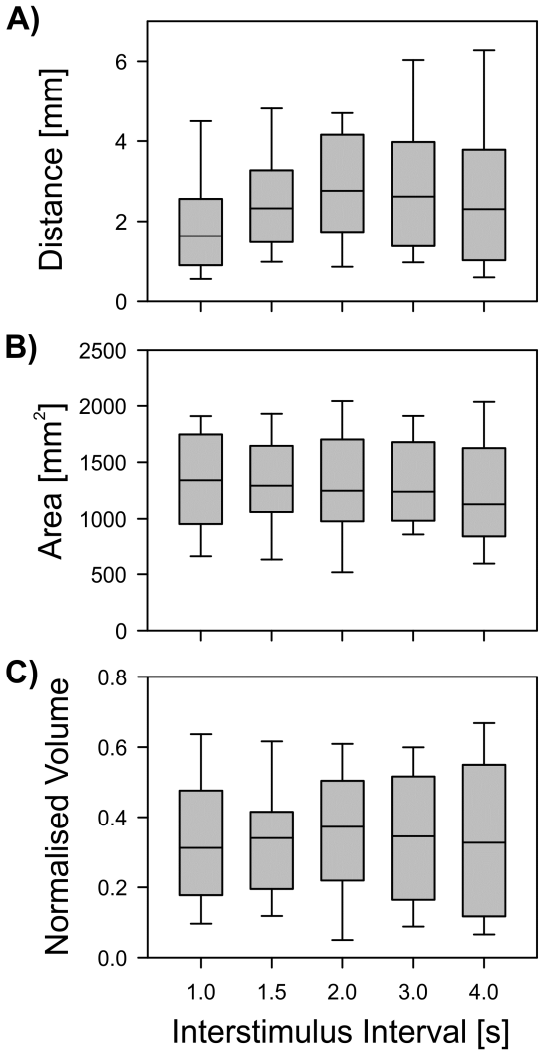
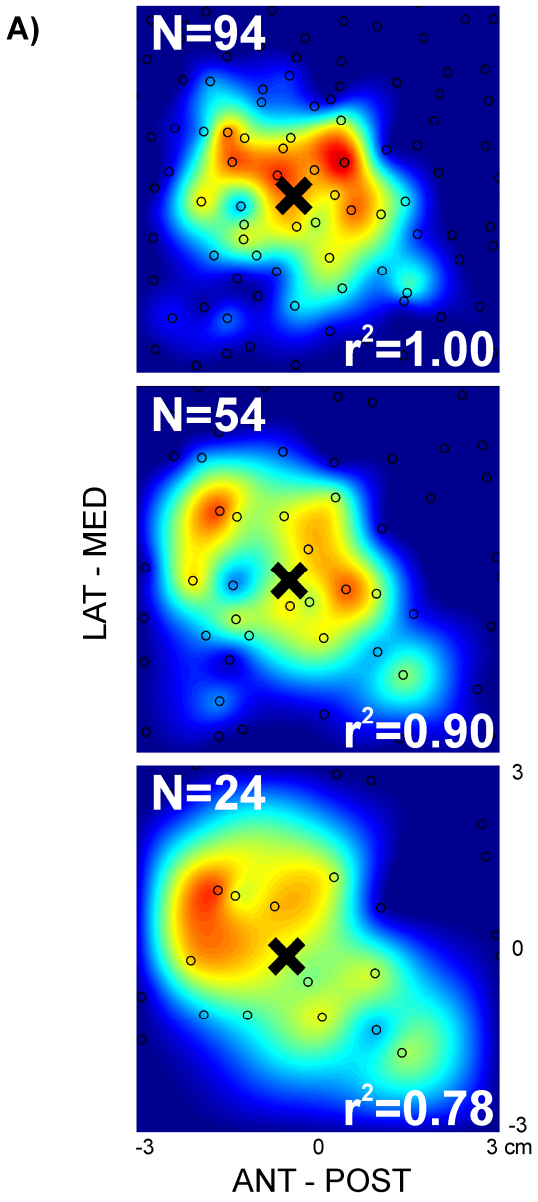
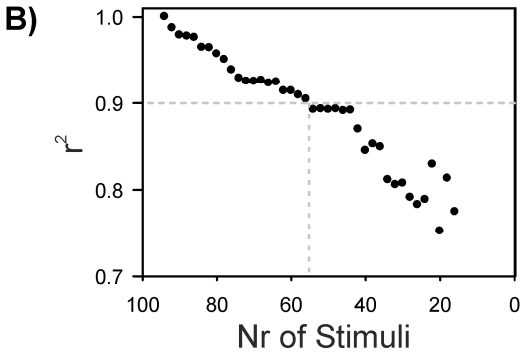
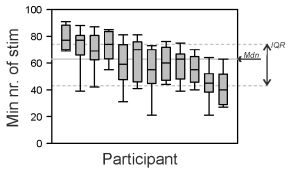
0.1
Normalised MEP$_{pp}$

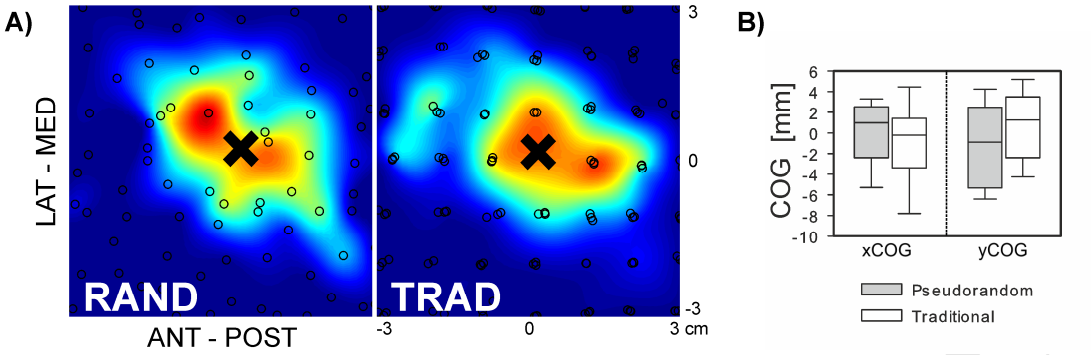












Highlights

- TMS maps are created using a pseudorandom walk method
- An interstimulus interval of 1 s can be used to acquire data for a TMS map
- Reliable TMS maps are created with as few as 63 stimuli
- TMS maps can be acquired in less than two minutes

Supplementary material:

Data acquisition: Collecting the EMG and neuronavigation data

Data acquisition for the TMS maps is started after determining the hotspot and motor threshold. Frameless stereotaxy (BrainSight 2, Rogue Research Inc, Montreal, Canada) was used to define a 6 x 6 cm grid as indicated by blue markers (see Figure 1A – right panel).The position and trajectory of each stimulus was illustrated on the display immediately after it was acquired. Experimenters were instructed to use this feedback to adjust coil position and orientation whilst stimuli were delivered at a constant intestimulus interval (typically 1.5 s). Moreover, experimenters were instructed to attempt to ensure the stimuli were equally spread across the grid, and not too stimulate twice in close proximity. The resulting grid of data was most consistent if the first four stimuli were delivered close to the blue corner markers of the grid. Thereafter, the procedure continued by pseudorandomly stimulating across the 6 x 6 cm square, with the location of successive stimuli determined by the experimenter.

Data analysis: How the map is created

Figure 1 in the main article illustrates how the EMG and neuronavigation data are used to construct a corticospinal excitability map. Maps were created offline with a bespoke MATLAB script **(**MATLAB Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States). For all EMG recordings the MEP was quantified by its peak-to-peak (MEP_{pp}) value, which was extracted from a window 20—50 ms after the stimulation (Figure 1A). The corresponding stimulation position in 3D space was extracted from the neuronavigation data. BrainSight makes use of the Polaris Vicra optical tracking system (NDI Medical, Ontario, Canada), which has an accuracy of 0.5 mm.

Three different coordinate systems were defined enabling transformation of the data from MRI coordinates to real world coordinates. The output data from the neuronavigation system includes a transformation matrix relating the orientation and position of every stimulation site to a global, MRI based, reference coordinate system (CSref).

$$BrainSight_{out} = \begin{bmatrix} X_{ref} & X \cdot x & X \cdot y & X \cdot z \\ Y_{ref} & Y \cdot x & Y \cdot y & Y \cdot z \\ Z_{ref} & Z \cdot x & Z \cdot y & Z \cdot z \end{bmatrix} \quad (1)$$

Stimulation position (X_{ref}, Y_{ref}, Z_{ref}) is expressed relative to the origin of CSref (x, y, z) located in the bottom left corner of the MRI (frontal view). Thereby, the x-axis runs parallel to the mediolateral axis, the y-axis parallel to the dorsoventral axis and the z-axis parallel to the superoinferior axis. A coil-based local coordinate system (CScoil; X, Y, Z) was used to determine the orientation of each stimulus. The stimulus position is given in millimetres while the orientations are expressed as direction cosines (in radians) representing the angles between the different axes. A third coordinate system generated from the cloud of position data represents the orientation of a plane fitted through all stimulation positions (CSFit) (Figure S A|B).

CSFit was determined by fitting a rectangular plane through the cloud of 3D position data. Using the assumption that every z-coordinate is functionally dependent on it's respective x and y-coordinate (x, y, f(x,y)), the fitting function is defined as:

$$\hat{Z}_{ref} = AX_{ref} + BY_{ref} + C \quad (2)$$

The plane fit was created using a least squares algorithm optimising a three parameter (A, B, C) error function:

$$Plane\_Fit(A,B,C) = \sum_{i=1}^{NrStim} \left[\left(AX_{ref,i} + BY_{ref,i} + C\right) - Z_{ref,i}\right]^2 \quad (3)$$

This hyperparaboloid function is solved by finding the combination of parameters (A,B,C) which give the minimum error between $\hat{Z}_{ref}$ and Z_{ref}. This corresponds to the combination of parameters where the integrated error function leads to a zero gradient in x, y and z:

$$\nabla E = \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix} = 2 \sum_{i=1}^{NrStim} \left[\left(AX_{ref,i} + BY_{ref,i} + C\right) - Z_{ref,i}\right] \begin{bmatrix} X_{ref,i} \\ Y_{ref,i} \\ 1 \end{bmatrix} \quad (4)$$

Written in matrix form, the equation becomes:

$$\begin{bmatrix} \sum X_{ref,i}{}^2 & \sum X_{ref,i} \cdot Y_{ref,i} & \sum X_{ref,i} \\ \sum X_{ref,i} \cdot Y_{ref,i} & \sum Y_{ref,i}{}^2 & \sum Y_{ref,i} \\ \sum X_{ref,i} & \sum Y_{ref,i} & 1 \end{bmatrix} \begin{bmatrix} A \\ B \\ C \end{bmatrix} = \begin{bmatrix} \sum X_{ref,i} \cdot Z_{ref,i} \\ \sum Y_{ref,i} \cdot Z_{ref,i} \\ \sum Z_{ref,i} \end{bmatrix} \quad (5)$$

This is an easily solvable three parameter (A, B, C) equation. The best fit plane is then solved by inputting the resulting parameters A, B and C input to equation 2 (Figure SC). These parameters were only determined once for each mapping session, using the first map data collected. Consequently, CSFit was expressed as the direction cosines matrix to CSref and used to define the orientation of the fitted plane. All position data were then transformed from 3D space to a 2D plane centred on the origin of CSref. An extra rotation was performed if the sides of the grid were not aligned with the X and Y axes of CSref (Figure S D).

Triangular linear interpolation was used to calculate an approximant that was subsequently used to create a full surface map within the transformed plane. This was calculated using the 'gridfit' MATLAB function [1]. This function uses a plane that is deformed using non-linear least squares methods to best fit the data. Two settings determine how this plane is transformed to best fit the data. The sensitivity (stiffness) of the plane defines how sensitive it is to rapid changes. The gridfit function allows for sensitivity range between 1-10. Using pilot data, we chose to use a sensitivity value of 2 as this afforded high sensitivity for rapid changes without over smoothing the variability. In addition, the function uses an interpolation density (step size) that defines the number of points with which the fitted value is approximated based on the acquired data. The grid was divided into 2500 partitions (50×50), with each point being assigned an approximated MEP value (aMEP) based on the nearest acquired MEP data (Figure SE). The result is a 2D representation of the corticospinal excitability akin to a contour plot (Figure 1B). A 3D corticospinal excitability map is also created using aMEP on the Z-axis (Figure 1B). In order to compare maps between participants, the colour bar was normalised to the minimum and maximum MEP value within a session.

Figure S approximately here

Exclusion criteria

Before the data was fitted with the rectangular plane and transformed to the origin of the CSref coordinate system, individual stimuli within a map were excluded based on four predefined criteria:

- RMS of background EMG

  RMS value of 45 ms EMG (50 – 5 ms preceding stimulation) was calculated for each individual EMG record. Mean and *SD* of all RMS values were then calculated and used to exclude EMG recordings exceeding mean + 2 *SD*. To limit the amout of data excluded by excessive backround EMG, feedback was provided to the participant about their level of EMG during the experiment.

- Position in 3D and 2D

  As the plane fit (Equation 3) was needed to transform the data from 3D to 2D, any outliers would worsen the fit and result in an inaccurate transformation. Therefore, to avoid stimuli outside the predefined grid affecting the plane fitted through the stimuli positions an initial transformation from 3D to 2D in CSref was calculated using the grid's orientation matrix as derived from the output of the neuronavigation software (Equation 1: $BrainSight_{out}$). Subsequently, all stimulation positions exceeding the sides of the grid by more than 20 mm in either X or Y when transformed to the origin were excluded from further analysis. This value was chosen based on pilot testing. Next, all data were transformed back to 3D to determine the plane fit according to Equation 3. After transformation to a 2D plane using the fitted plane, any stimuli exceeding the sides by more than 10 mm away were also excluded. In this case, 10 mm was used as it was found that stimuli delivered near the border of the grid as observed in BrainSight were usually found just outside the predefined grid when projected in a 2D plane. Accordingly, stimuli outside the grid but within 10 mm were

included and the grid enlarged. However, the same grid size was used for all maps in a participant; therefore grid sizes differed slightly between, but not within, participants.

- Extreme MEP outliers

MEP values exceeding mean + 3.5 *SD* of all MEP values within a map were excluded to avoid skewing the map based on a single MEP. As this criteria might be closely correlated with background EMG it was checked how many stimuli of the stimuli excluded on this criteria were also excluded based in the background EMG criteria. In total 55% of the stimuli excluded based on this criteria was also excluded based on a too high background EMG.

- Angle and translation relative to skull surface

The positioning of the TMS coil relative to the scalp is important to reduce MEP variability [2, 3]. Therefore the coil angle and translation relative to the scalp were used for exclusion. A single quadratic 3D surface was fitted through obtained neuronavigation data, to represent the skull. Best fit was determined for the transformed data in CSref:

$$\hat{Z} = A_1 + A_2 X_{ref} + A_3 Y_{ref} + A_4 {X_{ref}}^2 + A_5 {Y_{ref}}^2 + A_6 X_{ref} Y_{ref} \quad (6)$$

Translation and angle of each stimulus was determined relative to the fitted surface. Translation was expressed as the distance between the fitted surface Z-coordinate ($\hat{Z}$) and the actual stimulus Z-coordinate (Z_{ref}). The angle was calculated using BrainSight$_{out}$ to extract the CScoil. Thereby the direction of each axis of the coil is known (X_{coil}, $Y_{coil,}$ Z_{coil}). We also calculated the perpendicular axis (Z_{scalp}) to the derivatives in x and y direction of CSref at the stimulation location (X_{ref},Y_{ref}) of the quadratic 3D surface fit. Calculating the angle between Z_{scalp} and Z_{coil} gives a comparable measure for coil orientation relative to the scalp. Exclusion was based on the translation or angle falling outside the 99 % prediction interval.

In addition to taking precautions to reduce map variability, the TMS map was made less sensitive to MEP variability by the algorithm used to create the map. It has been suggested that the relative variability of MEPs near the border of the map is larger than the variability associated with MEPs recorded closer to the hotspot, and that this is the main source of the observed COG variability [4, 5]. Moreover, Brasil-Neto et al. [6] suggested more stimuli should be delivered at positions further away from the hotspot in order to achieve equal maximum error in determining the MEP_{pp} value at these positions. Both problems are reduced by the adopted method of creating a map. A plane is fitted through all acquired data; with a stiffness setting that determines the flexibility of the surface (see Supplementary Material for further detail). The stiffness setting of the fitted surface prevents skewing of the fitted plane as a result of greater variability in the periphery and thereby reduces the sensitivity of the map parameters to this local variability. In addition, in contrast to Brasil-Neto et al. [6] we suggest that using this method of creating the map it is possible to use fewer stimuli in the periphery and more near the 'hotspot', in order to achieve a higher spatial resolution in this most excitable area.

In total 8.2% of all stimuli were excluded before analysing the maps (180 maps analysed). Most stimuli were excluded due to high background EMG (4.2%) or angle and translation of the stimulus with respect to the skull (3.3%). For each map between 5 – 11 (8 ± 3) stimuli were excluded based on these predefined criteria.

Map parameters

Traditionally, the map area is defined by the number of excitable scalp sites and their distribution, typically a 1-cm spaced grid, with multiple stimuli per site [7]. In the present study, a map was created using a fixed grid size and by stimulating at random positions. A map was constructed from the grid position and EMG records by approximating the MEP size for 2500 partitions within the 6 x 6 cm grid. The map area was calculated by taking the ratio of the number of approximated partitions where the approximated MEP exceeded

153 10% of maximum approximated MEP ($aMEP_{10\%}$) relative to all partitions (N_{total} = 2500). This
154 method is based on Uy et al. [5], who demonstrated that the 10% cutoff reduces the
155 variability of the area by excluding the small variable MEPs near the boundaries of the map.

$$area = \frac{N\ (aMEP_{10\%})}{N_{total}} \times area_{map}$$

156 Where $area_{map}$ is the total mapped area of 36 cm^2.

157 Accordingly, map volume was the sum of all $aMEP_{10\%}$, subtracted by the 10% level. The
158 volume was normalised to the maximum volume found in all maps acquired during a single
159 session.

$$volume = \frac{\sum aMEP_{10\%} - 0.1 \times N\ (aMEP_{10\%}) \times aMEP_{max}}{MaxVolume}$$

160 COG is an amplitude weighted mean position of the map [7].

$$xCOG = \frac{\sum(x\ \cdot aMEP)}{\sum aMEP}$$

$$yCOG = \frac{\sum(y\ \cdot aMEP)}{\sum aMEP}$$

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Figure legends

Figure S: This figure highlights how the neuronavigation data is processed to create a 2D TMS map. (A) Three coordinate systems are used with x, y and z direction indicated by the green, blue and red arrow respectively. First, a global MRI based coordinate system (CSref) wherein all stimulation position is defined. Two local coordinate systems are used, one coil based (CScoil) to determine coil orientation and (B) one calculated (CSFit) based on a rectangular plane fitted through the data that contains the position of each stimulation administered. This plane fit is used to transform all neuronavigation from 3D to a 2D plane. (C) To align the grid with the X and Y axis of CSref an extra rotation of the transformed fitted plane is performed. Subsequently, every stimulus is matched with the from the EMG extracted peak-to-peak value of the MEP (D) To create the map an approximant is used to fill all 2500 (50 x 50) partitions of the grid based on the nearest acquired MEP data.