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Stokes MG, Barker AT, Dervinis M, Verbruggen F, Maizey L, Adams RC, Chambers CD. Biophysical determinants of transcranial magnetic stimulation: effects of excitability and depth of targeted area. *J Neurophysiol* 109: 437–444, 2013. First published October 31, 2012; doi:10.1152/jn.00510.2012.—Safe and effective transcranial magnetic stimulation (TMS) requires accurate intensity calibration. Output is typically calibrated to individual motor cortex excitability and applied to nonmotor brain areas, assuming that it captures a site nonspecific factor of excitability. We tested this assumption by correlating the effect of TMS at motor and visual cortex. In 30 participants, we measured motor threshold (MT) and phosphene threshold (PT) at the scalp surface and at coil-scalp distances of 3.17, 5.63, and 9.03 mm. We also modeled the effect of TMS in a simple head model to test the effect of distance. Four independent tests confirmed a significant correlation between PT and MT. We also found similar effects of distance in motor and visual areas, which did not correlate across participants. Computational modeling suggests that the relationship between the effect of distance and the induced electric field is effectively linear within the range of distances that have been explored empirically. We conclude that MT-guided calibration is valid for nonmotor brain areas if coil-cortex distance is taken into account. For standard figure-of-eight TMS coils connected to biphasic stimulators, the effect of cortical distance should be adjusted using a general correction factor of 2.7% stimulator output per millimeter.

transcranial magnetic stimulation; motor threshold; phosphene threshold; cortical excitability; transcranial magnetic stimulation safety; distance-adjusted motor threshold; transcranial magnetic stimulation modeling

ACCURATE, NONINVASIVE MANIPULATION of human brain activity is a key goal in clinical and experimental neuroscience. Over the last 25 years, transcranial magnetic stimulation (TMS) has emerged as a viable method for noninvasive stimulation of the human brain (Barker et al. 1985). Based on the principles of electromagnetic induction, TMS can deliver a relatively focal electric current to targeted areas of cortex via a time-varying magnetic field that passes unimpeded through the scalp and skull. Yet despite the important role of TMS in fundamental and applied neuroscience, there remains considerable uncertainty about how best to optimize stimulation protocols.

Safe and effective brain stimulation depends on accurate control over the level of induced neural excitation. Most importantly, overstimulation increases the risks associated with TMS, particularly the incidence of seizure (Rossi et al. 2009; Wassermann 1998). Overstimulation also reduces the focality of TMS (Brasil-Neto et al. 1992; Thielscher and Kammer 2004), thereby complicating the interpretation of experimental effects. Understimulation, on the other hand, reduces the likelihood of detecting significant experimental effects and could also compromise treatment in therapeutic settings (Kozel et al. 2000; McConnell et al. 2001; Mosimann et al. 2002; Nahas et al. 2001). Finally, quantitative comparisons between brain regions could be confounded by higher levels of cortical excitation at one brain site relative to another (Stokes et al. 2005, 2007).

Some studies have simply used a fixed stimulator output to set stimulation intensity. However, to provide greater control over cortical excitation, stimulator output is often calibrated to a measured estimate of motor cortex excitability, such as the threshold for eliciting a motor-evoked potential (Rossini et al. 1994) or overt twitch (Varnava et al. 2011). For studies of motor cortex, motor threshold (MT) provides a direct estimate of the effect of brain stimulation at the target site; subsequent stimulation can then be expressed as a percentage of MT. Stimulation of visual cortex also elicits a reliable response: a transient visual percept known as a phosphene (Abrahamyan et al. 2011b). Therefore, as in M1, stimulation threshold in visual cortex can be estimated by this reliable index of cortical effect (Aurora et al. 1998) and subsequent stimulation parameters can then be expressed as a percentage of phosphene threshold (PT; e.g., Abrahamyan et al. 2011a; Pascual-Leone and Walsh 2001).

Since calibration requires a reliable measure of the effect of TMS, such thresholding procedures are limited to brain areas in which a clear response to TMS can be measured after each pulse. Therefore, there is no direct way to calibrate TMS output for regions that do not yield a simple and/or reliable response to a single pulse. In practice, stimulator output is often calibrated to MT or PT even when applied to "silent" brain areas, but this practice assumes that a common threshold applies across sites. Previously, we questioned this basic assumption by showing that different cortical areas will receive different levels of stimulation depending on variations in distance between the targeted cortical surface and the stimulating coil (Cai et al. 2011; Stokes et al. 2005, 2007). We proposed a simple linear metric to correct for differences in coil-cortex distance; however, distance-adjusted MT still assumes that for different regions of the brain there is a common factor of cortical

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excitability. Here, we now examine this assumption by considering individual differences in MT and PT.

If excitation threshold measured at one cortical site reflects a general estimate of cortical excitability that is applicable to other cortical sites, then we expect MT and PT to correlate across individuals. Importantly, a number of previous studies have applied the same logic, but with mixed results (Antal et al. 2004; Boroojerdi et al. 2002; Deblieck et al. 2008; Gerwig et al. 2003; Stewart et al. 2001). However, close examination of these early studies shows that they were either statistically underpowered (Antal et al. 2004; Boroojerdi et al. 2002; Stewart et al. 2001) or failed to use consistent thresholding procedures (Gerwig et al. 2003). More recently, Deblieck et al. (2008) revealed that with sufficient statistical power and consistent thresholding approaches, a significant relationship between MT and PT is evident.

The first aim of this study was to replicate the results of Deblieck et al. (2008). Second, we also examined the distance effect for PT and MT, thus extending our previous studies that demonstrate a strong linear relationship between distance and stimulation threshold (Cai et al. 2011; Stokes et al. 2005, 2007). Finally, we also conducted a simulation study to further explore the key parameters that determine the relationship between the known generated magnetic field and the induced electric fields in underlying cortex.

MATERIALS AND METHODS

Participants

Thirty right-handed volunteers were recruited (13 male, 17 female; ages 19–39 yr, 24.9 ± 4.7 mean ± SD). All participants provided written informed consent and were screened for contraindications to TMS (Rossi et al. 2009; Wassermann 1998). The study was approved by the local Research Ethics Committee at the School of Psychology, Cardiff University.

Apparatus

Single pulses (<0.5 Hz) of TMS were delivered via a Magstim Rapid2 system and 70-mm figure-eight induction coil. For each participant, we also acquired high-resolution (1 × 1 × 1 mm) structural images using a GE 3T magnetic resonance imaging (MRI) scanner. Before scanning, contrast markers (vitamin E capsules) were attached to known scalp locations to enable subsequent TMS/MR coregistration using a magnetic tracking device (mini-BIRD 500, Ascension Tech) and MRIcro/MRIreg interface software (Rorden and Brett 2000). Scalp-coil distance was manipulated within participants using custom-machined acrylic separators measuring 3.17, 5.63, and 9.03 mm.

Procedure

MTs and PTs were obtained for each participant on separate days, and the order of acquisition was counterbalanced. The procedure was matched as closely as possible for both types of threshold estimation.

Motor threshold. Initially, the optimal stimulation site was localized in each participant using a standard "hunting" search procedure (Brasil-Neto et al. 1992). The primary motor cortex (M1) was localized in the right hemisphere by varying the precise position of the stimulating coil over the motor cortex while magnetic pulses were delivered at 60–80% of stimulator output. Throughout the hunting procedure and subsequent threshold estimation, the coil was oriented ~45° laterally from the midline with the handle pointing caudally; the initial flow of the induced biphasic current was therefore in a posteriolateral-anteriomedial direction. The site that produced the most robust muscle twitch was then marked on the scalp surface to ensure that the same coil position and orientation was used throughout the MT procedure, and also to enable subsequent coregistration with individual MRIs for localizing the stimulation site on the underlying cortical surface. The coil was held in place by hand, and the distance spacers were taped to the flat side of the coil. The coil position was maintained with reference to the markings drawn to the scalp surface during the initial hunting procedure.

After coil positioning, MT was obtained at the scalp surface (MT_0) and using the three distance separators ($MT_{3.17}$, $MT_{5.63}$, and $MT_{9.03}$). The order of the four distances (0, 3.17, 5.63, and 9.03 mm) was randomized. As described previously (Stokes et al. 2005, 2007; Varnava et al. 2011), resting MT was defined as the lowest stimulator output required to elicit an observable twitch in the contralateral hand muscle (abductor pollicis brevis) on 5 of 10 pulses. The threshold was estimated using a descending adaptive staircase procedure. For each condition, stimulation intensity was initially set to an above-threshold level and then reduced in steps of 5% until the response failed to reach the criterion. Stimulation was then increased in steps of 2% until the motor response was observed at above-threshold frequency. Finally, intensity was then reduced in steps of 1% until the response dropped below threshold again. The penultimate stimulator output was recorded as the threshold estimate. We have previously demonstrated that motor activity, defined by either observation of movement or electromyographic (EMG) recordings, produces reliable (Varnava et al. 2011) and highly correlated estimates of MT (Stokes et al. 2005).

Phosphene threshold. The general procedure for PT estimations was directly analogous to that described above for MT. Again, we used a hunting procedure to identify the site of maximal stimulation in visual cortex. The stimulating coil was initially positioned 1 cm above the inion and then varied in location while single pulses were administered at 60–80% stimulator output. Participants were blindfolded and asked to report whether or not they observed a phosphene, described to them as a brief distortion of their visual field. After reporting an initial phosphene, participants were given a number of additional above-threshold pulses to familiarize them with the target percept. The coil orientation was held constant with the handle pointing toward the right such that the induced current initially flowed in a right-left direction. After localization of an optimal occipital site for inducing phosphenes, the scalp position was marked so we could maintain the same position throughout the thresholding experiment and to allow subsequent MR coregistration after testing.

Thresholds were obtained at the scalp surface (PT_0) and using the three distance separators ($PT_{3.17}$, $PT_{5.63}$, and $PT_{9.03}$), randomized within participants. We also used an adaptive staircase to estimate the lowest TMS intensity required to induce a phosphene on at least 5 of 10 pulses. Participants were instructed to adopt a conservative threshold and to respond "yes" only if they were certain and to say nothing if no phosphene was perceived. Since there can be no objective measure of phosphene induction, we also considered the possibility that PTs might be less reliable than MTs. The relationship between PT and our distance manipulation was highly reliable (mean $R^2 = 0.95$; see RESULTS for further details), which can be interpreted as a conservative estimate of the reliability for each individual estimate: the variance explained by the fitted regression depends directly on the variance of the estimates and can never exceed it. Nevertheless, to assess more specifically the test/retest reliability of PT (independently of our experimental manipulation), we performed the full PT procedure twice in the majority of participants during the same testing session. The results of the second PT estimation confirmed the reliability of PT (see RESULTS). Thus, despite the subjective nature of phosphene perception, these results demonstrate that PT is highly reliable and reproducible. This reliability corresponds closely with the observed reliability of MT (Varnava et al. 2011).

Cortex-scalp distance calculation. To estimate the distance between the scalp surface and underlying cortical surface, we used a

customized procedure for analyzing high-resolution structural MRIs for TMS applications (Cai et al. 2011; Varnava et al. 2011; Matlab code available for download at http://the-brain-box.blogspot.co.uk/DistanceCode). First, the surface of the cortex was estimated by fitting a mesh model of the gray matter, segmented using routines from SPM 8 (Wellcome Department of Cognitive Neurology, London, UK). Next, we calculated the three-dimensional distance between each cortical voxel and the scalp location that was stimulated during the MT and PT estimations. Stimulation coordinates were transformed to voxel space using miniBIRD/MRIreg. For a robust estimate of scalp-cortex distance, we calculated the mean distance of the 100 nearest cortical voxels. This automated procedure is relatively fast; the most time-consuming steps are typically the normalization and segmentation steps in SPM.

Field modeling. The induced electric fields were modeled with the electromagnetics finite element package SEMCAD 14.6 (Schmid & Partner Engineering, Zurich, Switzerland). A simple head model was chosen, consisting of a realistic head shape (SAM: standard anthropomorphic head shell model) with an assumed skull thickness of 14 mm (i.e., the gap between the scalp and the cortex), filled with a uniform conducting material to simulate a homogeneous brain. The head volume was meshed with 11.1×10^6 voxels of dimensions $1 \times 1 \times 1$ mm. The figure-eight coil was simulated as nine circular loops of wire in each wing within a nonconducting housing of overall thickness of 12 mm, the turns being positioned at the same radii as in the actual coil. The fields were calculated using the SEMCAD quasi-static low-frequency solver with a coil current frequency of 4 kHz, which approximates to that from the stimulator used in this experiment (Magstim Rapid2), and an arbitrary peak coil current of 5 kA (Bijsterbosch et al. 2012). Because a quasi-static solver has been used here, neither the current waveform frequency nor its chosen amplitude will affect the spatial distribution of induced field in the head model. Although the absolute magnitudes of the induced field will depend on these values, this study only considers the relative values for different coil positions and at different positions in the head.

RESULTS

Correlation Between PT and MT

First, we determined the relationship across participants between estimates of MT and PT (Fig. 1). We observed a significant correlation between MT and PT at all levels of the manipulated distance: $r(MT_0, PT_0) = 0.51$, $P = 0.004$; $r(MT_{3.17}, PT_{3.17}) = 0.38$, $P = 0.041$; $r(MT_{5.63}, PT_{5.63}) = 0.51$, $P = 0.004$; $r(MT_{9.03}, PT_{9.03}) = 0.54$, $P = 0.004$. These relationships indicate that individual differences in cortical excitability in M1 are significantly related to estimates of cortical excitability in visual cortex.

Previously, we demonstrated that MT is highly reproducible, with test/retest reliability over $r = 0.95$ (Varnava et al. 2011). The current data further demonstrate the reproducibility of PT. The significant correlations described above between PT at various distances and the independent measure of MT represents a lower-bound estimate of the reliability of both MT and PT. For a more direct estimate, the cross-correlation between PT estimates at the four distance manipulations ranged from $r = 0.918$ to $r = 0.951$. Finally, in a subset of participants ($n = 28$), we also performed the full PT distance manipulation twice. Test/retest reliability between corresponding conditions ranged from $r = 0.932$ to $r = 0.95$. Altogether, these findings indicate a strong relationship between these two reliable methods for estimating excitability of motor and visual cortex.

Effect of Distance: Group Result

Next, we explored the relationship between scalp-coil distance and PT/MT (Fig. 2*A*). A two-way ANOVA, with factors for threshold type (MT, PT) and scalp-coil distance (0, 3.17, 5.63)[1] revealed significant main effects of threshold type ($F_{1,29} = 31.9$, $P < 0.001$) and scalp-coil distance ($F_{2,58} = 281.0$, $P < 0.001$). There was also a significant two-way interaction ($F_{2,58} = 5.3$, $P = 0.008$), indicating that the effect of distance depended on the threshold measure. Previously, we found that the effect of distance depends on the absolute MT_0 (Stokes et al. 2007): participants with a higher overall MT showed a greater effect of distance (see also Cai et al. 2011; Varnava et al. 2011). In the present study, we have shown that PT is higher than MT; therefore, the interaction between the effect of distance between the two measures of cortical excitability could reflect this dependency on absolute threshold.

We and others have also shown previously that individual differences in MT correlate with the measured distance from the scalp surface to underlying motor cortex (Kozel et al. 2000; McConnell et al. 2001; Stokes et al. 2005, 2007). In this study, we again found a significant correlation between MT and measured scalp-cortex distance at the site of stimulation ($r = 0.47$, $P = 0.009$). However, we did not identify a similar relationship for PT ($r = 0.04$, $P = 0.82$). This null result could reflect variation in the depth of the critical region for generating a phosphene in each participant (Thielscher et al. 2010).

Effect of Distance: Individual Estimates

To explore the effect of distance in each participant, we calculated the linear fit relating distance and threshold within each participant. All participants exhibited a strong positive relationship between threshold and distance (PT: mean $R^2 = 0.91$; range 0.72–0.99; MT: mean $R^2 = 0.97$; range 0.67–0.99). PT gradients (PT_g) ranged from 1.0 to 4.7% output per millimeter [mean = 2.8%, ±0.29 95% confidence interval ($CI_{95\%}$)], and MT gradients (MT_g) ranged from 0.9 to 3.0% (mean = 2.2%, ±0.15 $CI_{95\%}$). As implied by the interaction between the effect of distance and threshold measure reported above, the slope of these linear fits was significantly higher for PT compared with MT ($t_{29} = 4.2$, $P < 0.001$).

There was no significant correlation between PT_g and MT_g across participants ($r = 0.2$, $P = 0.29$); however, there was a significant relationship between individual estimates of slope and overall threshold [$r(MT_0,MT_g) = 0.495$, $P = 0.005$; $r(PT_0,PT_g) = 0.484$, $P = 0.007$; Fig. 2, *B* and *C*]. This finding replicates existing evidence that the distance effect depends positively on the baseline stimulation threshold (Cai et al. 2011; Stokes et al. 2007; Varnava et al. 2011). Previously, we have suggested that this dependency could be due to individual differences in the relative size of the relevant functional area (Stokes et al. 2007).

Field Modeling

It has been consistently shown that despite the induced electric field strength declining nonlinearly with distance, the relationship between distance and TMS effect is well characterized by a linear function over the range of practical interest

[1] Scalp-coil distance 9.03 mm was excluded due to several missing data points for PT at this level (threshold > 100% stimulator output).

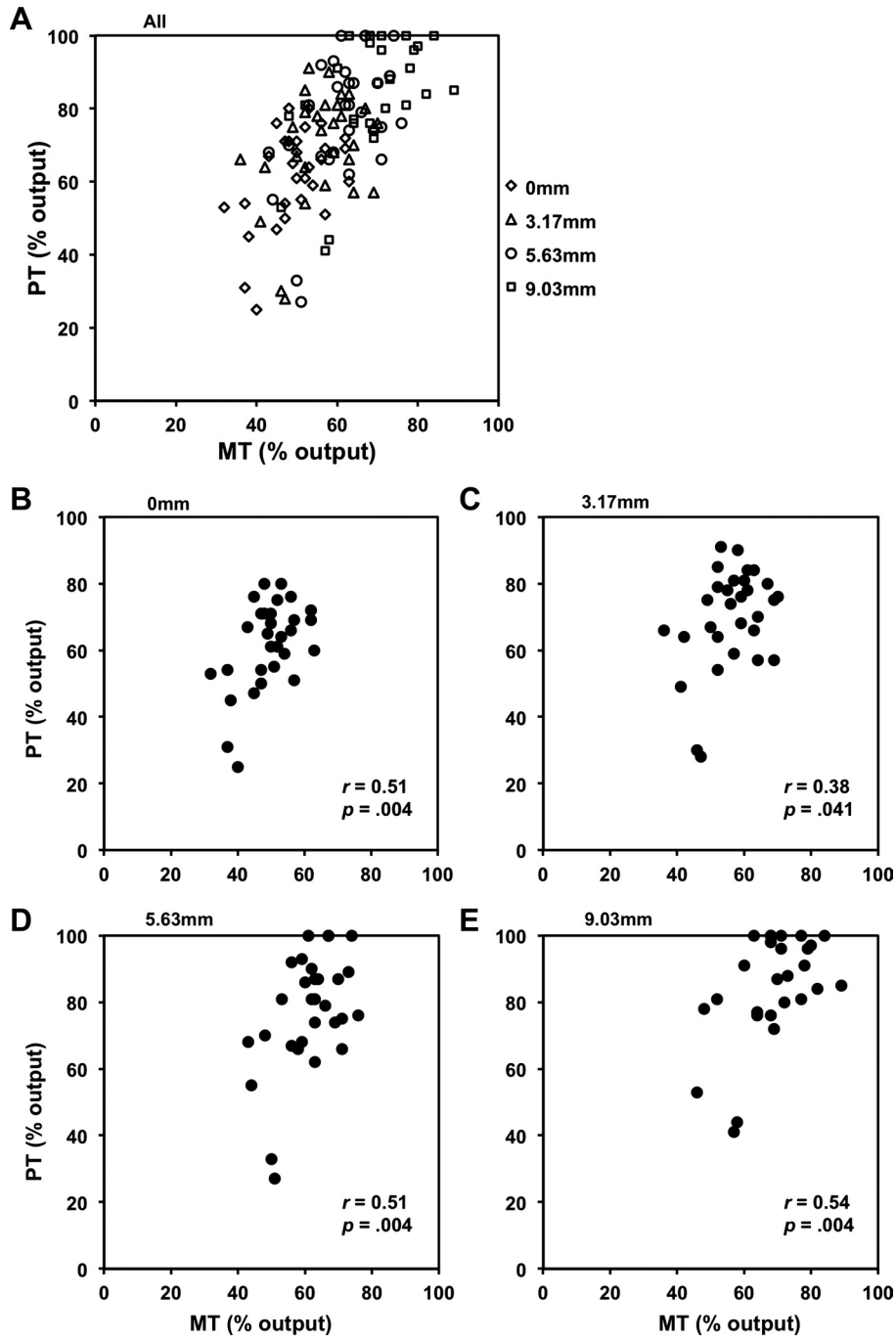

Fig. 1. Relationship between individual estimates of motor threshold (MT) and phosphene threshold (PT). *A*: each data point represents the MT and PT measured in the same participant at 4 levels of scalp-coil distance: 0, 3.17, 5.63, and 9.03 mm. *B–E*: the same results as in *A* plotted separately for each scalp-coil distance. A significant positive correlation was obtained in each case.

(Cai et al. 2011; Kozel et al. 2000; McConnell et al. 2001; Stokes et al. 2005, 2007). The magnetic field generated by a figure-eight coil is complex; therefore, to estimate induced electric field at various distances between the coil and cortical surface, we used a realistic head model. We found that the relationship between coil-scalp distance and the induced electrical field can be characterized by a linear function, at least within the range between 0 and 10 mm (Fig. 3*C*).

DISCUSSION

The results of this study demonstrate a significant relationship between MT and PT. In each of four separate tests we have shown that individual differences in PT are coupled to MT. We also found that the effect of distance is similar for MT and PT. In both cases, threshold values increase linearly with increasing distance between the scalp and stimulating coil. Importantly, there was no significant relationship between the gradient of the distance effect measured for PT and MT; however, both effect gradients correlated significantly with the overall threshold value.

General Excitability Factor

These results provide evidence for a general factor of cortical excitability within participants. This is a key assumption for MT-guided TMS protocols. If cortical excitability at one brain area generalizes to other brain areas, then any measure of

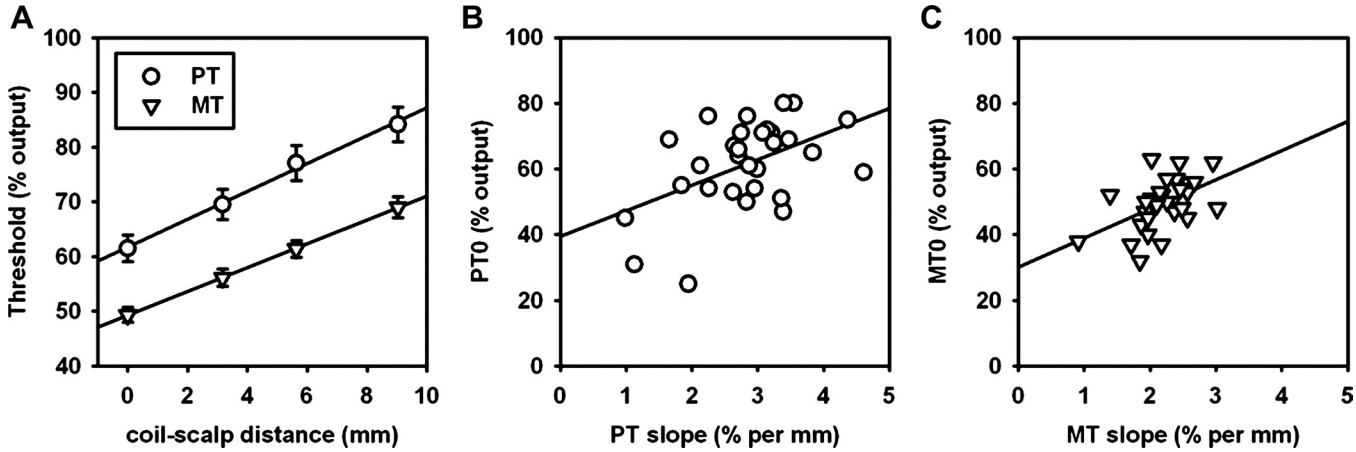

Fig. 2. Effect of distance on PT and MT. *A*: average PT and MT are plotted as a function of the manipulated coil-scalp distance. Lines represent the regression of PT or MT on to coil-scalp distance. Error bars are ±SE. *B*: individual differences in PT at the scalp surface (PT_0) are plotted as a function of individual differences in the distance-effect slope for PT. The line represents the relationship between PT and slope. *C*: the corresponding plot for MT_0 and MT slope.

excitability can be meaningfully applied to calibrate stimulation parameters at other sites for which measures of cortical excitability are unavailable. To date, the majority of TMS studies have used MT to calibrate output for silent brain areas (Rossi et al. 2009), under the assumption that MT will generalize to nonmotor areas. However, a number of empirical studies previously questioned this key assumption (Antal et al. 2004; Boroojerdi et al. 2002; Gerwig et al. 2003; Stewart et al. 2001). By comparing individual differences in MT with PT, it is possible to test whether these two local measures of excitability capture a common factor of excitability for each participant. Surprisingly, early studies failed to identify a strong link between MT and PT, questioning the assumption that threshold measured at one site generalizes to other cortical sites.

However, closer inspection of these previous studies reveals a number of factors that limit their conclusions. First, Stewart et al. (2001), Boroojerdi et al. (2002), and Antal et al. (2004) were underpowered for correlation analyses. In particular, Boroojerdi et al. (2002) included only 8 participants, whereas Antal et al. (2004) tested 11 participants and Stewart et al. (2001) tested 15. Clearly, these samples are too small to confidently assert a null correlation between MT and PT. Moreover, each of these studies reported moderate correlation coefficients, ranging from 0.3 (Stewart et al. 2001) to 0.4 (Boroojerdi et al. 2002). Under some conditions, Antal et al. (2004) even reported significant relationships between threshold measured in visual and motor cortex.

In contrast to these underpowered studies, Gerwig et al. (2003) tested 32 participants, yet still failed to identify a relationship between MT and PT. However, these researchers used different methods for estimating threshold in motor and visual cortices (for detailed discussion, see Deblieck et al. 2008). For MT, intensity was initially set above threshold and then lowed in steps of 2% until less than 5 of 10 pulses elicited a motor response. In contrast, PT threshold was estimated by varying intensity randomly in 2% steps. Offline thresholds were then defined as the minimum intensity that exceeded the threshold of 5 of 10 pulses to elicit a visual response. These different procedures would likely result in different threshold estimates even within the same brain region (Mills and Nithi 1997), thus undermining the ability to observe a correlation between different regions.

Recently, Deblieck et al. (2008) employed identical thresholding procedures for PT and MT, and revealed a significant

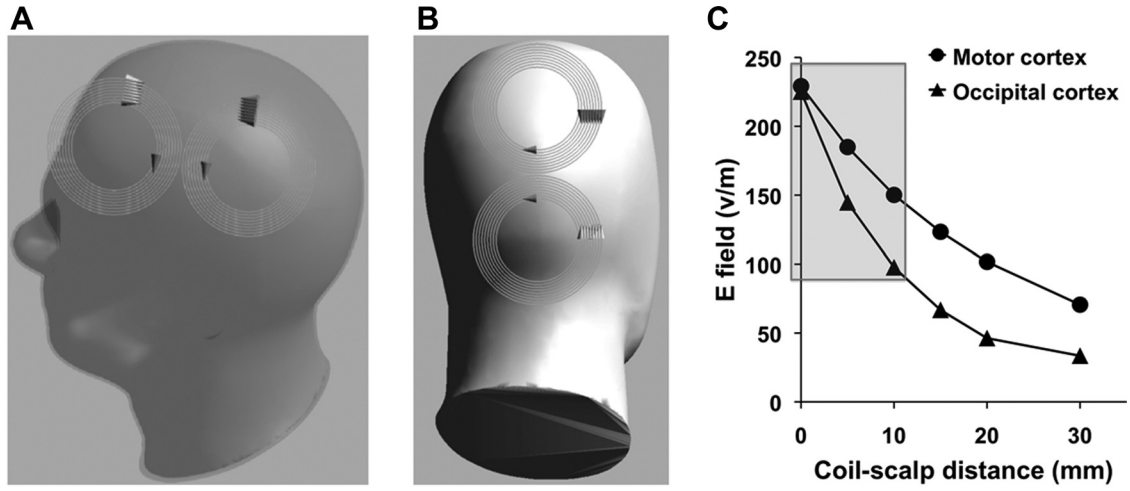

Fig. 3. Model of cortical stimulation as a function of scalp-coil distance. In the simulations, a 70-mm figure-eight coil was positioned over the motor cortex (*A*) and visual cortex (*B*) of a head model. *C*: estimated electric field strength (V/m) is plotted according to distance between the coil housing and motor/occipital scalp site. The shaded area indicates the approximate range of coil-scalp distances tested empirically in the current study (0–10 mm).

relationship across 27 participants. They argue that differences in threshold estimation procedures are likely to account for the lack of previous evidence for a relationship between MT and PT. Our results provide additional support for this conclusion and the assumption that threshold estimations vary according to a generalized factor of cortical excitability. Therefore, calibrating to a measurable estimate of cortical excitability can be expected to reduce interparticipant variation in cortical stimulation. It should be noted that certain disease states could be associated with inhomogeneous changes in cortical excitability. However, distance correction could still be important for improving patient safety by reducing output in circumstances that could otherwise risk overstimulation.

Effect of Distance

The relationship between PT and MT implies that the effect of TMS depends on a general factor of cortical excitability. However, we and others have previously demonstrated that the effect of TMS also critically depends on the distance between coil and cortical stimulation site (Kozel et al. 2000; McConnell et al. 2001; Stokes et al. 2005). To characterize the effect of distance on TMS, we developed a method to systematically vary scalp-coil distance over M1 and measure the effect on MT (Stokes et al. 2005). This procedure revealed a tight coupling between changes in scalp-coil distance and the percentage of stimulator output required to reach MT (Cai et al. 2011; Stokes et al. 2005, 2007). In the present study we extend our findings to visual cortex, revealing a strong relationship between changes in scalp-coil distance and PT. As with MT, this relationship is well characterized by a linear function within a coil-scalp distance range of 10 mm.

It is interesting to note that despite the highly reliable fit of the linear function within participants (overall mean $R^2 = 0.94$), we also observed substantial variation in the gradient of the distance effect. This variability is unlikely to reflect error in the slope estimation, because we found good test/retest reliability between slopes estimated within participants during separate sessions ($r = 0.74$). Rather, our data suggest that variations in the effect of distance are stable within a given site (i.e., an individual's visual cortex) but that unlike threshold, the slope parameter does not generalize across sites.

Finally, the simulation also provides important insights into the underlying function relating distance to TMS effects. As expected, the overall relationship is nonlinear; however, the portion of the curve that lies within the range that we have manipulated (0–10 mm) is well characterized by a linear function. This finding accords with previous observations that the effect of distance approximates linearity within this range of realistic distances (Stokes et al. 2005, 2007; Varnava et al. 2011).

Correcting for Distance

Controlling for the effects of scalp-cortex distance is critical when comparing threshold estimations between sites and/or participants. This can be achieved simply by using the distance-effect gradient to correct the observed threshold measure according to the measured distance between the targeted cortical surface and the overlaying scalp surface (Stokes et al. 2005). This approach increases the sensitivity of tests comparing threshold estimations between participants and, more importantly, could reduce the potential of artifactual results when comparing thresholds between groups that are likely to differ in scalp-cortex distance (e.g., as a function of sex, age, or clinical typology).

Within participants, it is particularly important to account for distance effects when using MT or PT to calibrate stimulator output for other cortical regions. If scalp-cortex distance is known both at the calibration location (e.g., M1) and the target site, and if the relationship between distance and TMS is known, then the simple formula can be applied:

$$\mathrm{AdjMT\%} = g \times (D_{\mathrm{Site}X} - D_{\mathrm{M1}}) + \mathrm{MT}_{\mathrm{M1}}, \qquad (1)$$

where AdjMT is the adjusted MT in %stimulator output, MT is the unadjusted MT in %stimulator output, D_{M1} is the distance between the scalp and M1, $D_{\mathrm{Site}X}$ is the distance between the scalp and a second cortical region (site X), and g is the distance-effect gradient. Our results suggest that the distance-effect gradient is not a general biophysical parameter within participants; therefore, individualized estimates at one cortical location are unlikely to generalize to others. Consequently, an estimate of the mean gradient across participants is more likely to be predictive of any particular gradient at an unknown stimulation site. The mean effect of distance can then be applied as a rule of thumb across participants to minimize the effect of distance. Using a biphasic Magstim system with a 70-mm figure-eight coil, we observed a mean gradient of 2.7%/mm across this and other published studies (Stokes et al. 2005, 2007; Varnava et al. 2011). This value is based on gradient estimations in 92 unique participants. To maximize the generalizability to other nonmotor brain areas, we assigned equal weighting in this calculation for gradients estimated using PT and MT. We thus recommend that future TMS studies employing a 50- or 70-mm figure-eight coil should correct for variation in scalp-cortex distance by using $g = 2.7$ in *Eq. 1* above.

It is important to note that different hardware configurations are likely to result in systematic differences in the effect of TMS. Previously, we found no difference between 50- and 70-mm figure-eight coils (Stokes et al. 2007); however, we did observe substantially lower distance-effect gradients for a "batwing" coil geometry that is optimized for stimulation of deeper brain areas (1.4%/mm in Cai et al. 2011). Therefore, it is important to determine the average distance-effect size gradient for new configurations.

Estimating Scalp-Cortex Distance

For calculating distance adjusted MT, it is important to have an accurate estimate of the scalp-cortex distances at the calibration site (e.g., M1) and the target stimulation site. Accurate measures of scalp-cortex require high-resolution structural MRIs; however, under some circumstances it might be difficult to back-reconstruct the precise location of the stimulation. Although we have repeatedly demonstrated a relationship between distance at M1 and individual differences in MT, in this study we were unable to detect the equivalent relationship between PT and distance over visual cortex. This null effect is most likely accounted for by variability in the cortical folding associated with visual cortex. Without knowing a priori the cortical location that gave rise to the visual response (cf. Thielscher et al. 2010), our best estimation was that, on

average, the critical site would be the cortical surface closest to the scalp. However, variation in the true locus of effect could easily obscure the relationship between PT and scalp-cortex distance. In light of this potential problem, we suggest that distance correction would be more accurate if based on MT, because the measurement of M1 distance more reliably predicts the observable effect of TMS (Kozel et al. 2000; McConnell et al. 2001; Stokes et al. 2005, 2007).

Distance correction also assumes accurate measurement of the scalp-cortex distance at the target nonmotor region. If the target site can be identified anatomically (e.g., according to sulcal landmarks), then distance can be estimated from structural MRIs (Ahdab et al. 2010; Herwig et al. 2001). If, however, the target site is defined according to functional MRI, distance is calculated relative to the scalp surface and the overlaid functional activation maps (Sack et al. 2009; Sparing et al. 2008). Error in measuring the distance to the target site is equivalent to localization error in other directions, all of which inevitably reduce the efficacy of the TMS. It is therefore recommended that in the absence of a direct functional assay (such as a phosphene or motor response), TMS should be guided by accurate neuronavigation using structural as well as functional MRI.

Finally, TMS is sometimes applied with reference to probabilistic localization protocols. For example, the stimulating coil may be positioned over a scalp location defined by the 10–20 international EEG coordinate system (Herwig et al. 2003). If probabilistic scalp-cortex distance estimations were also available within this reference frame, a probabilistic adjustment could help reduce the average effect of differences between site depths. Nevertheless, given substantial individual differences in scalp-cortex distance (Stokes et al. 2005), we strongly recommend that TMS intensity be calibrated according to individual anatomy (Sack et al. 2009).

We conclude that the effect of TMS depends on the intrinsic cortical excitability and coil-cortex distance. Our results suggest that cortical excitability is likely to be a stable parameter within participants; therefore, estimates of excitability such as PT and MT are useful for setting stimulation parameters for targeting other brain areas. The distance from scalp to functionally critical cortex is easier to estimate at M1 than in visual cortex; therefore, distance-adjusted MT is likely to be more reliable than distance-adjusted PT. Accurate estimates of distance to the target site depend directly on the accuracy of neuronavigation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.G.S. and C.D.C. conception and design of research; M.G.S., A.T.B., M.D., F.V., L.M., R.C.A., and C.D.C. analyzed data; M.G.S., A.T.B., M.D., F.V., and C.D.C. interpreted results of experiments; M.G.S. and C.D.C. drafted manuscript; M.G.S., A.T.B., M.D., F.V., L.M., R.C.A., and C.D.C. edited and revised manuscript; M.G.S., A.T.B., M.D., F.V., L.M., R.C.A., and C.D.C. approved final version of manuscript; A.T.B., M.D., F.V., L.M., R.C.A., and C.D.C. performed experiments; C.D.C. prepared figures.

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