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Simple Metric For Scaling Motor Threshold Based on Scalp-Cortex Distance: Application to Studies Using Transcranial Magnetic Stimulation

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Stokes, Mark G., Christopher D. Chambers, Ian C. Gould, Tracy R. Henderson, Natasha E. Janko, Nicholas B. Allen, and Jason B. Mattingley. Simple metric for scaling motor threshold based on scalp-cortex distance: application to studies using transcranial magnetic stimulation. *J Neurophysiol* 94: 4520–4527, 2005. First published August 31, 2005; doi:10.1152/jn.00067.2005. Transcranial magnetic stimulation (TMS) is a unique method in neuroscience used to stimulate focal regions of the human brain. As TMS gains popularity in experimental and clinical domains, techniques for controlling the extent of brain stimulation are becoming increasingly important. At present, TMS intensity is typically calibrated to the excitability of the human motor cortex, a measure referred to as *motor threshold* (MT). Although TMS is commonly applied to nonmotor regions, most applications do not consider the effect of changes in distance between the stimulating device and underlying neural tissue. Here we show that for every millimeter from the stimulating coil, an additional 3% of TMS output is required to induce an equivalent level of brain stimulation at the motor cortex. This abrupt spatial gradient will have crucial consequences when TMS is applied to nonmotor regions because of substantial variance in scalp-cortex distances over different regions of the head. Stimulation protocols that do not account for cortical distance therefore risk substantial under- or overstimulation. We describe a simple method for adjusting MT to account for variations in cortical distance, thus providing a more accurate calibration than unadjusted MT for the safe and effective application of TMS in clinical and experimental neuroscience.

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a noninvasive neurostimulation technique used widely in clinical and experimental neuroscience. By inducing a "virtual lesion" within the healthy human brain, TMS can identify which cortical regions are necessary for specific behavioral functions, including those involved in human perception and cognition (Pascual-Leone et al. 1999; Walsh and Cowey 2000). The unique capacity to explore the causal role of discrete cortical regions has established TMS as a valuable tool for experimental brain research, alongside neuroimaging techniques such as functional magnetic resonance imaging (Hallett 2000). In particular, TMS has been successfully applied to explore the functional neuroanatomy of cognition, including the time course of processing (Chambers et al. 2004a) and connectivity between different brain regions (Pascual-Leone and Walsh 2001). It is also emerging as a powerful therapeutic and diagnostic instrument in both neurological (Currà et al. 2002) and psychiatric (Pridmore and Belmaker 1999) settings.

During TMS, an induction coil placed over the scalp discharges a brief magnetic pulse. Unimpeded by the skull and scalp, the time-varying magnetic field induces a small electric current within the underlying cerebral cortex (Ruohonen and Ilmoniemi 2002). The induced current causes neurons to depolarize, resulting in a local increase in brain activity. However, for accurate, safe, and effective application of brain stimulation, it is essential that an appropriate level of electric current be induced within a target region. Understimulation reduces the probability of detecting significant experimental results and, within clinical domains, deprives patients of necessary treatment dosages (Mosimann et al. 2002). Overstimulation, on the other hand, reduces focality by enlarging the area of directly stimulated cortex (Roth et al. 1991) and increasing the likelihood of indirect transynaptic stimulation of distant brain structures (Paus et al. 1997). Overstimulation also increases the risk of adverse effects such as seizures (Wassermann 1998). At present, stimulation intensity is typically determined according to a measure of cortical excitability known as *motor threshold* (MT; see Fig. 1*A*). MT is defined as the minimum stimulation intensity applied to motor cortex (M1) required to induce a reliable motor response such as an electromyographic (EMG) response >50 μV (Rossini et al. 1994) or visible twitch in a predefined muscle of the contralateral hand (Pridmore et al. 1998). TMS protocols expressed as a percentage of MT allow stimulator output to be calibrated individually to an overt physiological response, even when applied to nonmotor cortical regions. Standardization of stimulation protocols is essential for comparison of TMS effects between participants and stimulator types and to establish safety guidelines (Wassermann 1998).

Although considered only rarely, the rapid decline in magnetic field strength with distance is a critical determinant of cortical stimulation (Ruohonen and Ilmoniemi 2002). If targeted cortical sites vary in depth from the stimulating coil, then the strength of the magnetic field, and thus the magnitude of cortical stimulation, will also vary (Fig. 1*B*). Indeed, individual differences in MT are closely related to variations in the cortical depth of M1 (Kozel et al. 2000; McConnell et al. 2001). Consequently, when the intensity of TMS applied to nonmotor regions is based on MT, variations in depth from the overlying scalp surface will result in different levels of effec-

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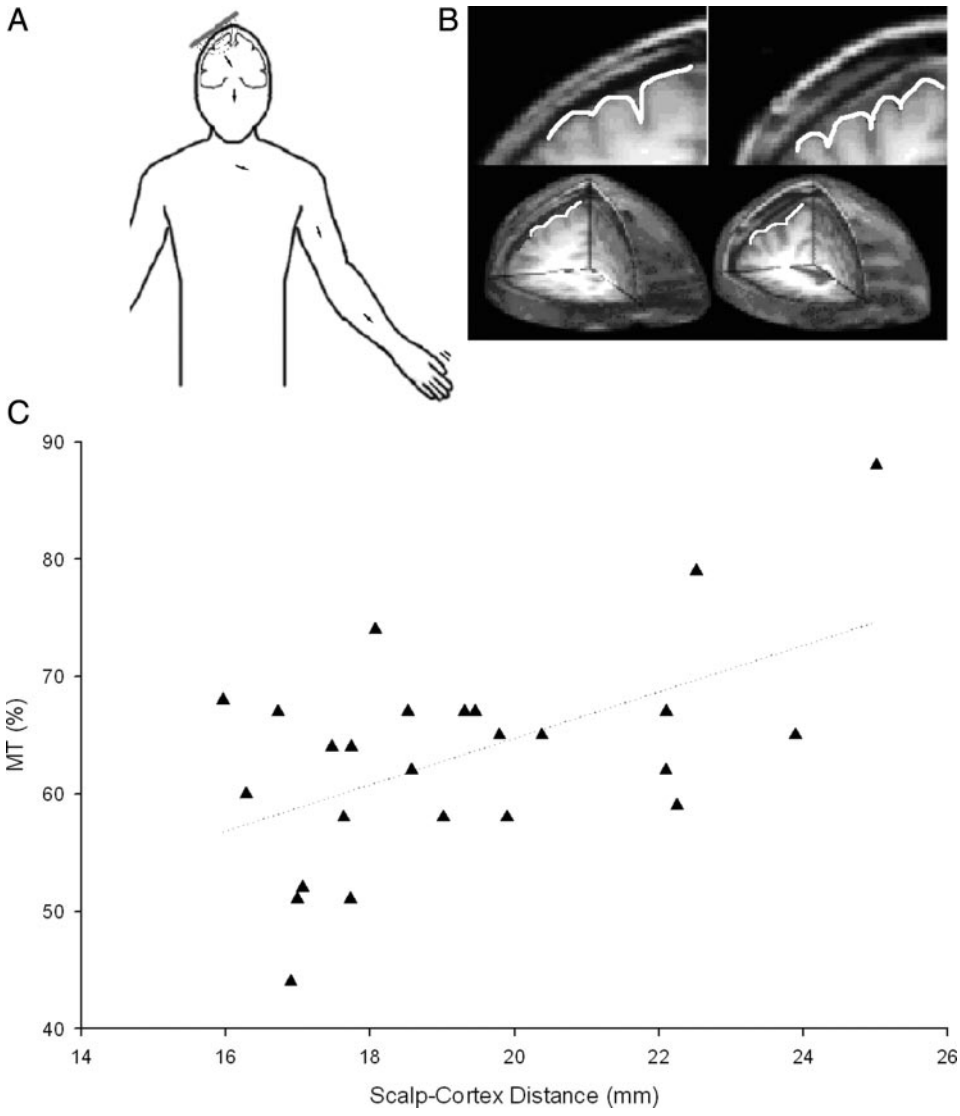

FIG. 1. Greater cortical depth yields a reduction of effective brain stimulation. *A*: motor threshold (MT) provides a convenient measure of the cortical effect of transcranial magnetic stimulation (TMS). Stimulation applied to the scalp overlying motor cortex (M1) induces overt motor activity in the contralateral hand muscle that can be identified visually (Pridmore et al. 1998) or recorded using an electromyogram (Rossini et al. 1994). MT is defined as the minimum percent of stimulator output required to reliably induce a motor response. *B*: structural magnetic resonance imaging (MRI) scans reveal substantial variation in the cortical depth of M1 between individuals. The white line indicates the cortical surface of M1. *C*: effect of individual variations in the depth of M1 was revealed by comparing observed MT with the scalp-cortex distance measured at M1. Each data point represents an individual participant, and the straight line represents a linear regression. As illustrated, individuals with greater scalp-cortex distances tend also to have a higher MT.

tive stimulation. Such distance-dependent TMS effects at nonmotor regions have been observed using fMRI (Nahas et al. 2001) and in response to therapeutic administration (Mosimann et al. 2002).

Despite the known influence of cortical distance on brain stimulation, no standard method has yet been established that accounts for variations in coil distance. One suggested approach has been to adjust MT according to the exponential decline in magnetic field strength with distance (Nahas et al. 2004); however, magnetic field strength is just one of several parameters that determine the effect of distance on cortical excitation (Ruohonen and Ilmoniemi 2002). Theoretical descriptions must also account for complex interactions between a variety of parameters, including the shape of the magnetic field, as well as the local conductivity and total area of the stimulated tissue (Jalinous 1991; Miranda et al. 2003). Here we report a novel approach to establishing the relationship between coil distance and MT. We show that increasing the distance between the TMS induction coil and the cortex results in a systematic decrease in effective stimulation of motor cortex. We describe a simple linear correction to provide a more reliable MT-based protocol for nonmotor TMS application.

METHODS

Participants

Thirty-three healthy adults (18 males; 15 female, aged 18–40, 22.6 ± 3.97; mean ± SD) gave written informed consent and were paid for their participation. All were right-handed by self-report, and all were screened prior to testing for contraindications to TMS (Wassermann 1998). Experiments were approved by the University of Melbourne's Human Research Ethics Committee.

Apparatus

A 2.2T biphasic MagStim Rapid system was used to deliver TMS pulses (60-μs magnetic field rise time, 250-μs pulse duration) via a standard 70-mm figure-eight induction coil (MagStim). Prior to TMS testing, a T1-weighted MR scan was obtained for each participant using a GE Signa 3T system (1.3 × 1.3 × 1.3 mm, sagittal acquisition). As described previously in detail (Chambers et al. 2004b), individual MRIs were then co-registered to the participants' head using a magnetic tracking device (miniBIRD 500, Ascension Tech)

and MRIcro/MRIreg interface software (Rorden and Brett 2000). The distance between the scalp and stimulating coil was manipulated using custom-machined acrylic plastic sheets ranging from 1 to 10 mm in thickness. In a supplementary experiment, a Grass Model 12 Neurodata acquisition system connected to an IBM compatible computer via a PC-Labcard 812-PG analogue to digital converter was used to measure TMS-evoked motor potentials. Raw EMG signals were amplified using a Grass 12A5 AC amplifier with an amplification factor of 50,000 and half-amplitude high- and low-frequency cutoffs set at 1 kHz and 30 Hz, respectively. Evoked motor responses were acquired and processed using the VPM (version 11.6) (Cook 1995) software package, and were sampled at 1 kHz from 500 ms before the onset of the TMS pulse and until 500 ms after probe onset.

Procedure

As in previous studies (Kozel et al. 2000; McConnell et al. 2001), MT was defined in the primary experiment as the minimum stimulator output required to induce a visible twitch in the abductor pollicis brevis (APB) on 5 of 10 consecutive pulses delivered at a rate of $\leq$1 Hz to the motor cortex. For each participant, the virtual cathode was positioned over M1, and the lowest stimulator output to induce a motor response was determined using an adaptive staircase method. The location of M1 was determined by varying the position of the coil over the scalp in the right hemisphere until a reliable twitch in the APB was observed. For later reference, this location was then marked on the scalp using a semi-permanent marker. Stimulator intensity was increased after trials in which an APB response was present on less than five out of ten trials (step sizes of 10 and 2%), and decreased for trials in which an APB response was present on $\geq$5 of 10 (step sizes of 5 and 1%). MT was thus set as the percentage of maximum stimulator output that produced a reliable motor response.

To investigate the effect of coil distance on brain stimulation, we initially examined the relationship between individual differences in MT and corresponding variations in the depth of M1. The distance between the scalp location of TMS and the underlying motor cortex was determined using MRIcro software. As described by McConnell et al. (2001), the average distance between the scalp and cortical surface was calculated across a region spanning 16 voxels (~21 mm) in the coronal plane by 7 voxels (~9 mm) in the sagittal plane, centered around, and perpendicular to, the scalp surface identified as the position of the virtual cathode.

Coil-scalp distance was then systematically varied within individual participants to isolate the effects of distance on the cortical response to TMS. With the stimulating coil positioned tangential to the scalp surface, the plastic spacers were placed between the coil and the scalp while maintaining the alignment of the virtual cathode over the marked scalp location to ensure the same cortical stimulation site irrespective of scalp-coil distance. The order in which MT was obtained for each spacer was randomized. In our initial investigation, all 10 spacers were used, whereas the abbreviated version used only the 5- and 10-mm spacers.

Finally, to determine the practical implications of distance-related changes in effective stimulation observed at M1, cortical depths were determined for a range of nonmotor sites. Neuroanatomical locations were identified in slice and three-dimensional-rendered MRIs, and the distance from cortex to scalp surface was calculated as the distance between the target cortical site and the voxel representing the closest scalp surface determined in all three axes.

RESULTS

Relationship between MT and scalp-cortex distance

As shown in previous studies (Kozel et al. 2000; McConnell et al. 2001), we found that participants with greater distance between scalp and cortex required higher levels of stimulator output to induce a reliable motor response (Fig. 1*C*). A regression analysis performed on the between-participant data confirmed a significant linear relationship ($y = 2.0x + 25.2$) between MT and scalp-cortex distance [$R^2 = 0.29$; $F(1,23) = 9.3$, $P = 0.006$], accounting for 29% of the variance.

Relationship between MT and coil-scalp distance

Within-participant examination of distance related effects confirmed the relationship between the coil-cortex distance and the level of effective brain stimulation. Figure 2*A* shows data from one representative participant, revealing a monotonic increase in MT as the coil is moved outward from the scalp surface. All 25 participants showed a similar relationship between coil-scalp distance (19.3 $\pm$ 2.3) and MT (63.9 $\pm$ 6.5; all $P < 0.004$). The mean gradient for the regression lines was 2.9 $\pm$ 0.3%/mm with an average constant of 7.6 $\pm$ 10.4. On average, this relationship accounted for 96 $\pm$ 2.2% of the variance.

Although the within-participant data were extremely well characterized by a linear function, it is possible that the range of distances tested within each participant encompassed only a relatively monotonic segment of an otherwise nonlinear function. If so, we might still expect to find a systematic relationship between the observed gradient and the starting point of the range of samples. In contrast, however, we found that individual gradients did not correlate significantly with the distance between scalp and cortex ($r = 0.213$, $P = 0.428$). Furthermore, the data pooled across participants (Fig. 2*B*) also revealed a significant linear relationship ($y = 2.2x + 24.3$) between MT and estimated coil-cortex distance [$R^2 = 0.61$, $F(1,166) = 261$, $P < 0.001$].

To determine the within-participant effect of coil distance on MT independently of absolute coil-cortex distance, the *change* in MT was analyzed for each increment in scalp-coil distance (Fig. 2*C*). The regression performed on pooled data accounted for 90% of the variance ($y = 2.9x - 0.367$; $R^2 = 0.90$, $P < 0.001$). Finally, a linear regression and trend analysis were performed on the average change in MT for each distance increment (Fig. 2*D*). This regression ($y = 2.9x - 0.3$) accounted for >99% of the variance ($R^2 = 0.99$, $P < 0.001$), and the trend analysis identified an exclusive linear component [$F(1,10) = 1,359$, $P < 0.001$; $P > 0.15$ for all higher-order components]. Overall, within-participant analyses identified a gradient of ~3%/mm, thus demonstrating that for each millimeter between the cortex and induction coil, an additional 3% of absolute stimulator output is required to induce an equivalent neural response in the motor cortex.

Variations in cortical distance demonstrate the importance of scaling brain stimulation

To illustrate the practical implications of distance-related TMS effects, MRI scans from 18 participants were analyzed to determine scalp-cortex distances at various commonly targeted stimulation sites (Fig. 3*A*). A two-way repeated-measures ANOVA with factors of anatomical site and hemisphere revealed a significant main effect of anatomical site [$F(8,136) = 55.6$, $P < 0.001$], but no other significant terms (all $P > 0.4$). Bonferroni-corrected post hoc comparisons were undertaken between each site, collapsed across hemisphere. As shown in

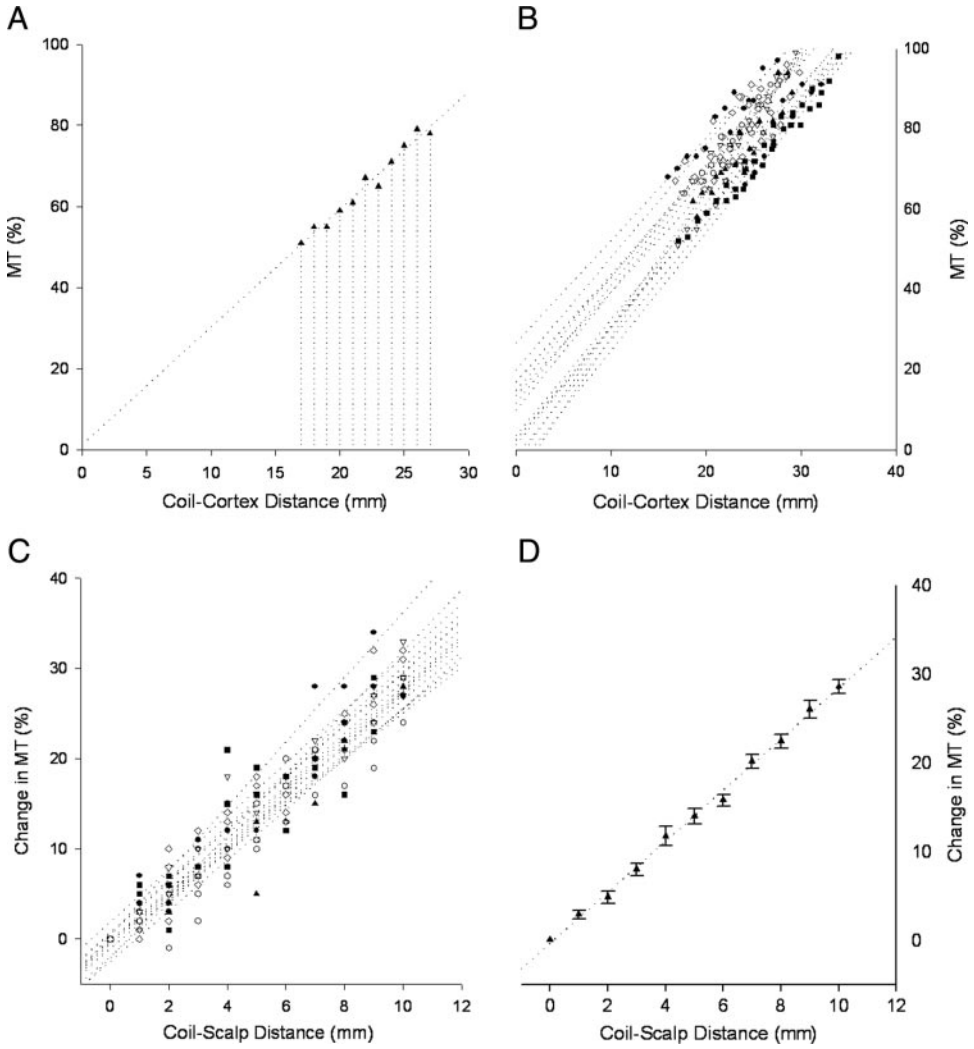

FIG. 2. The relationship between distance and cortical stimulation was examined by measuring MT at various distances between the coil and scalp. In each participant, the distance from scalp to stimulating coil was incremented randomly in units of 1 mm.*A*: results of 1 representative participant are shown as a function of MT and coil-cortex distance, starting from scalp-cortex distance. The regression line ($y = 2.9x + 1.3$) indicates the linear component to the relationship [$R^2 = 0.97$, $F(1,9) = 299.4$, $P < 0.001$]. *B*: all participants exhibited a similar positive relationship between MT and coil-cortex distance. *C*: change in MT associated with each increment of scalp-coil distance was calculated to evaluate the distance-related effects independently from baseline differences in MT and/or error in M1 distance estimations. Results are shown separately for each participant. *D*: mean change in MT across participants was calculated for each coil-scalp increment. Error bars represent ±1 SE.

Table 1, the cortical distances for almost all sites differed significantly from M1, and many nonmotor sites differed from each other. Figure 3*B* illustrates the variation in scalp-cortex distance projected on to a three-dimensional, surface-rendered MRI for a representative participant. The color-coded scale representing the scalp-cortex distance shows that inferior brain regions such as temporal and occipital cortex, as well as inferior subregions of the frontal cortex, are generally closer to the scalp surface than more superior sites such as the superior parietal lobule (SPL) and M1. These variations highlight the need to account for cortical distance when calibrating the intensity of TMS.

Abbreviated method for scaling brain stimulation

Having demonstrated the importance of coil distance, we also assessed the effectiveness of an abbreviated method for determining the relationship between cortical distance and MT. An identical procedure was performed on a further nine participants, with MT obtained at three different scalp distances. This abbreviated version resulted in an equivalent average gradient (2.9 ± 0.58), and a slightly higher average linear fit ($R^2 = 0.97$; $\Delta R^2 = 0.04$, $P = 0.024$). Again, no significant correlation was observed between scalp-cortex distance and gradient ($r = 0.097$, $P = 0.81$).

Relationship between muscle twitch and EMG recordings

Induced motor activity is typically measured either visually according to muscle twitches or using EMG recordings (Rossini et al. 1994). Although previous research has shown that both methods provide a reliable measure of motor activity (Pridmore et al. 1998), the precise relationship between these two common techniques has not previously been examined in detail. Here, we sought to investigate the relationship between a continuous measure of the peak-to-peak amplitude of evoked muscle activity and binary measures derived according to alternative threshold criteria: overt muscle twitch and evoked muscle activity over 50 μV. Stimulator intensity was randomly varied between ±3% of twitch-defined MT (MT-3%, MT-2%, MT-1%, MT, MT +1%, MT +2%, MT +3%) while muscle activity was recorded visually and via EMG in 10 participants. EMG data were analyzed by peak-to-peak amplitude, and according to a binary rating of whether the evoked muscle activity exceeded a threshold of 50 μV. All three measures correlated highly with the seven perithreshold TMS intensities (twitch $r = 0.99$, $P < 0.001$; EMG $r = 0.88$, $P = 0.01$; $EMG_{50\mu V}$ $r = 0.93$, $P = 0.003$). A two-way ANOVA performed on the factors of TMS intensity (−3, −2, −1, 0, 1, 2, 3) and measure type (muscle twitch, EMG, $EMG_{50\mu V}$) revealed main effects of TMS intensity [$F(6,54) = 11.1$, $P <$

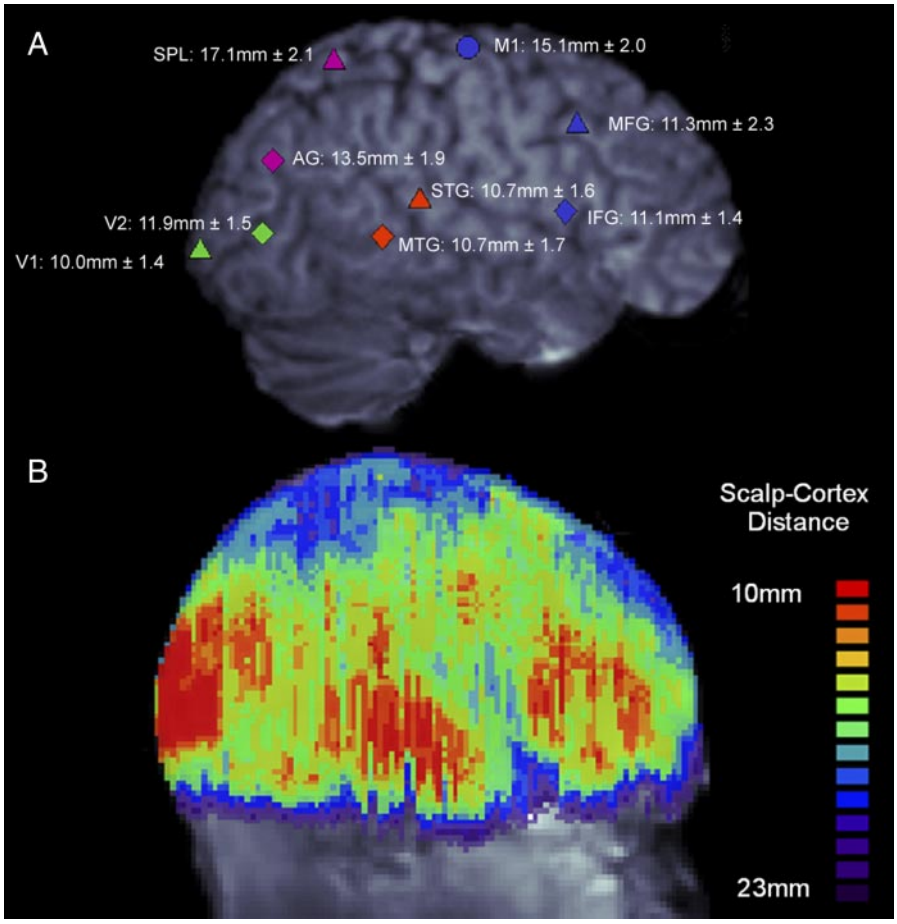

FIG. 3. The depth of underlying cortex varies across the surface of the scalp. *A*: scalp-cortex distance was measured across a range of brain regions. Mean ± SD of the distances between scalp and cortex are shown for each site. Frontal sites (blue): primary motor cortex (M1/BA4: circle), middle frontal gyrus (MFG/BA9: triangle), inferior frontal gyrus (IFG/BA45, diamond). Temporal sites (red): superior temporal gyrus (STG/BA22: triangle), middle temporal gyrus (MTG/BA 21: diamond). Parietal sites (purple): superior parietal lobule (SPL/BA7: triangle) and angular gyrus (AG/BA39: diamond). Occipital lobe sites (green): primary visual cortex (V1/BA17: triangle) and secondary visual cortex (V2/BA18: diamond). [B] Scalp-cortex distances for each voxel representing the scalp surface, shown in the right hemisphere of one participant. Distance is color-coded, with red representing small distances, and blue/purple representing large distances.

0.001] and measure type [mean muscle twitch = 16%, mean $EMG_{50\mu V}$ = 23%; $F(2,18) = 8.1$, $P = 0.001$]. The interaction term was not significant [$F(12,108) = 2.0$, $P = 0.103$]. Trend analyses revealed an exclusive linear component for twitch [$F(1,9) = 40.0$, $P < 0.001$], EMG [$F(1,9) = 2.5.0$, $P = 0.033$] and $EMG_{50\mu V}$ [$F(1,9) = 10.0$, $P = 0.012$].

TABLE 1. *Structural MRI analysis of cortical depths for a range of commonly stimulated brain regions*

	Frontal		Parietal		Temporal		Occipital	
	MFG	IFG	SPL	AG	STG	MTG	V1	V2
Frontal								
M1	3.8**	4.0**	−2.0*	1.7	4.4**	4.5**	5.2**	3.2**
	0.48*	0.21	0.52*	0.54*	0.54*	0.13	0.29	0.28
MFG	—	0.2	−5.8**	−2.1*	0.6	0.7	1.4	−.5
		0.38	0.66**	0.55*	0.56*	0.32	0.43	.48*
IFG	—	—	−5.9**	−2.3*	0.5	0.5	1.2	−0.7
			0.34	0.42	0.48*	0.50*	0.39	0.12
Parietal								
SPL	—	—	—	3.6**	6.4**	6.4**	7.1**	5.2**
				0.77**	0.28	0.16	0.53*	0.20
AG	—	—	—	—	2.8**	2.8*	3.5**	1.6*
					0.53*	0.32	0.60*	0.55*
Temporal								
STG	—	—	—	—	—	<0.1	0.7	−1.2*
						0.77**	0.54*	0.73**
MTG	—	—	—	—	—	—	0.7	−1.2*
							0.43	0.64*
Occipital								
V1	—	—	—	—	—	—	—	−1.9*
								.48*

Upper entries denote the mean difference in scalp-cortex distance (mm) between sites (x_i-x_j), and lower entries are the respective correlation values. MRI, magnetic resonance imaging; MFG and IFG, middle and inferior frontal gyrus; SPL, superior parietal lobe; AG, angular gyrus; STG and MTG, superior and middle temporal gyrus; V1 and V2, primary and secondary visual cortex. *$P < 0.01$, ** $P < 0.001$.

DISCUSSION

The present findings demonstrate the importance of distance between the source of stimulation and cortical surface during TMS. The effect of distance on motor cortex stimulation is characterized by a steep linear relationship between MT and depth of stimulated cortical tissue. The practical implications of applying MT-based TMS to nonmotor regions were shown by structural MRI analyses that revealed significant variation in the depth of cortical sites, implying significant distance-related effects in nonmotor TMS applications.

Initially, we found that individuals with a greater scalp-cortex distance tended also to have higher MTs. These distance-related effects are consistent with known properties of magnetic field distributions generated in TMS (Ruohonen and Ilmoniemi 2002) and suggest that individual differences in cortical excitability indexed by MT may be largely accounted for by differences in skull and scalp thickness (Kozel et al. 2000; McConnell et al. 2001). It is possible that extraneous influences such as individual differences in cortical excitability, or perhaps even underlying nonlinearities (Kozel et al. 2000; McConnell et al. 2001) may have resulted in substantial unexplained variance. However, it is also likely that much of the unexplained variance was due to inaccuracies in localizing the precise region of M1 that was stimulated with TMS. Although TMS/MRI co-registration provides an accurate method for identifying cortical structures underlying the scalp surface (Chambers et al. 2004a,b; Herwig et al. 2001), fMRI evidence suggests that the depth of hand representations within M1 varies considerably between individuals (Wassermann et al. 1996b).

To isolate the effects of coil-cortex distance at M1, we systematically varied the distance between the stimulating coil and scalp surface within individual participants. Linear regression analyses provided an excellent fit of the relationship, accounting for between 91 and 99% of the variance. The average slope of the regression line was 2.9, indicating that for every millimeter increment in coil-scalp distance, ~3% additional stimulator output is required to induce an equivalent cortical effect at M1. The strong linear relationship between distance and MT was replicated across participants using the average change in MT calculated for each increment of distance. The regression line accounted for >99% of the variance and yielded the same estimate for the slope as the average individual results. Furthermore, we found that binary threshold measures based on a motor response recorded either as a muscle twitch, or an EMG amplitude >50 μV, each produce a reliable measure of TMS effect as did a continuous measure of EMG activity. Although the flux density of a magnetic field declines nonlinearly, it is interesting to note that we found a consistent linear relationship between distance and cortical effect at M1. It is possible that the range of distances examined in the present study covered a linear segment of an otherwise nonlinear function. Furthermore, although flux density is an important determinant of induced electrical current, it is not the only relevant parameter that influences the level of electromagnetic induction within underlying cortex (Ruohonen and Ilmoniemi 2002). The shape of the magnetic field, which is determined by coil-geometry, also varies as a function of distance, resulting in a number of complex interactions between flux density, area of secondary conductor (cortex), and induced current. Indeed, it is possible that nonlinear relationships between area and density of magnetic field combine to approximate a linear function, at least within the distance range considered in the present experiment.

The results of our study therefore indicate that a simple linear correction can be applied to the commonly used MT-based expression of stimulator output to account for the effects of distance

$$\mathrm{AdjMT\%} = \mathrm{MT} + \mathrm{m} \times (\mathrm{D_{SiteX}} - \mathrm{D_{M1}})$$

where AdjMT% is the adjusted MT in percentage stimulator output, MT is the unadjusted MT in percentage stimulator output, D_{M1} is the distance between the scalp and M1, D_{SiteX} is the distance between the scalp and a second cortical region (SiteX), and *m* is the spatial gradient relating MT to distance. In cases where the spatial gradient is unknown, an approximate derivation of distance-adjusted MT can be obtained by replacing *m* with the average gradient obtained in the present study

$$\mathrm{AdjMT\%} = \mathrm{MT} + 3 \times (\mathrm{D_{SiteX}} - \mathrm{D_{M1}})$$

We explored the potential implications of applying unadjusted MT to nonmotor regions by calculating average scalp-cortex distances across a range of possible sites. The structural MRI analyses revealed substantial regional variation, with seven of the eight sites differing significantly from M1. In general, nonmotor cortical areas were closer to the scalp surface than M1, implying that the application of TMS according to *unadjusted* MT is likely to overstimulate the underlying cortex. Overstimulation reduces the focality of TMS and elevates the risk of adverse TMS effects such as seizures. Of particular concern is evidence that commonly targeted regions such as the dorsolateral prefrontal cortex (Wassermann and Lisanby 2001) were on average 4 mm closer to the scalp surface than M1. This observation shows that a stimulation protocol set at 120% of standard MT, when applied to the middle frontal gyrus (MFG), would be equivalent to *>150%* of distance-adjusted MT, thus exceeding recommended safety guidelines when delivered at typical frequencies and durations (Wassermann 1998). This change in effective stimulation intensity might explain previous cases of seizures during TMS applied to frontal regions within MT-based safety guidelines (Wassermann et al. 1996a). In particular, our findings show that safety guidelines must consider *individual* variations in cortical distances. From our sample of 18 participants, discrepancies of ≤16 mm were identified, thus clearly placing some individuals at an unacceptable risk unless MT is appropriately scaled.

As demonstrated in the present study, TMS investigations of M1 can exploit MT as an overt measure of cortical effects. Consequently, M1 studies provide an ideal basis for investigating the behavioral effects of intensity and other important stimulation parameters, including frequency and duration. For instance, qualitatively distinct cortical effects have been observed for stimuli presented above versus below MT (Kujirai et al. 1993). Critically, reducing the variance associated with changes in scalp-cortex distance will enable TMS protocols developed at M1 to be more accurately applied to other regions, thus allowing greater control of neural activity.

Although MT is currently the standard calibration method used for nonmotor stimulation, variations in cortical excitability due to differences in cytoarchitecture and corticocortical

connectivity will also influence the effect of TMS. Using distance-adjusted MT, future studies could examine systematic variations in cortical excitability across different regions. Furthermore, future studies might also examine the effect of distance at nonmotor sites to confirm the relationship identified in the present study. The methodological challenge for nonmotor studies, however, is to determine a reliable index of cortical stimulation. Although this presents a significant challenge for prefrontal and association cortex, primary sensory cortical regions might provide a more robust measurable response. For example, previous studies have used a measure of visual cortex excitability known as phosphene threshold (PT) to assess the validity of MT as a general measure of cortical excitability (Boroojerdi et al. 2002; Gerwig et al. 2003; Stewart et al. 2001). PT represents the minimum stimulator output over visual cortex required to induce a brief visual percept (e.g., flash of light), or phosphene, and is thus considered the visual analog of MT (Stewart et al. 2001). TMS studies that have failed to show a correlation between MT and PT have concluded that MT cannot be used to represent nonmotor regions due to intrinsic differences in neural tissue (Boroojerdi et al. 2002; Gerwig et al. 2003; Stewart et al. 2001). However, since scalp-cortex distance is a critical determinant of cortical sensitivity to TMS, a correlation between unadjusted MT and PT would also require a strong cross-correlation between the depth of M1 and primary visual cortex (Stewart et al. 2001). Although our structural brain analysis revealed that scalp-cortex distance was indeed correlated between some sites, almost half showed no significant correlation, including between M1 and V1. In the absence of a strong relationship between the depth of M1 and V1, future studies should consider the relationship between PT and distance-adjusted MT. Reducing the independent variation between the distances over the motor cortex and the occipital pole might allow a significant relationship between these two measures of cortical excitability to emerge. Clearly, the known effect of distance must first be controlled for before systematic differences in electric properties between cortical regions can be meaningfully compared using TMS.

In many experimental designs, quantitative comparisons are made between the effects of stimulating different cortical regions. Such comparisons are important because they can reveal functional dissociations between regions of interest (Chambers et al. 2004a) or control for nonspecific effects of TMS (Rushworth et al. 2001). However, in addition to differences from M1, we also found that the depth of several nonmotor cortical regions also differ from one another, even for subregions within the same lobe (e.g., AG and SPL in the parietal lobe; V1 and V2 in the occipital lobe). Consequent changes in effective stimulation could give rise to artifactual, but nonetheless statistically significant, quantitative differences. Therefore as a minimum requirement we suggest that the relative stimulation could be equated between sites to allow for quantitative contrasts. This can be achieved using the scaling co-efficient without reference to either M1 distance or MT

$$\text{Output}_{\text{site2}} = \text{Output}_{\text{site1}} + m \times (D_{\text{site2}} - D_{\text{site1}})$$

where Output $_{\text{site1}}$ and Output $_{\text{site2}}$ represent the percent of absolute stimulator output for Site 1 and Site 2, respectively, D_{site1} and D_{site2} represent their cortical distances, and m is the spatial gradient.

In considering the generality of the present findings, it is important to note that the properties of the magnetic field will differ according to variations in coil geometries. In particular, the magnetic field strength generated by smaller coil arrangements declines at a greater rate with distance (Ruohonen and Ilmoniemi 2002). The present study used a standard 70 mm figure-eight coil to determine parameters that are applicable to a wide range of laboratory and clinical settings (Jalinous 2002). However, it will be necessary for future studies to determine relevant scaling coefficients for use with custom coil designs. Our final investigation verified the accuracy of a more convenient method of establishing a distance-scaling factor. Using only three distance-varied samples of MT, the abbreviated method actually produced a slightly more reliable estimate of MT/distance relationship, presumably due to less overall cortical stimulation involved in the procedure. Accumulated effects of TMS are known to reduce cortical excitability in M1 (Chen and Seitz 2001), and because distance was randomized, changes in cortical excitability over time would add variance to MT that is unrelated to the effects of distance. Thus we recommend the application of a three-sample method to determine parameters for use with other coil geometries and possibly other stimulator types (e.g., monophasic).

TMS is gaining prominence as a powerful tool for demonstrating causal relationships between neural activity and behavior and is a promising therapeutic aid in psychiatric settings (Pridmore and Belmaker 1999). We have shown that the safe and effective application of TMS requires the depth of cortical sites to be considered when determining appropriate stimulation protocols. We suggest that distance-adjusted MT, as described here, can be used to establish more accurate stimulation protocols, to compare experimental findings, and to develop more effective treatment regimes.

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