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Efficient and reliable characterization of the corticospinal system using transcranial magnetic stimulation

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Abstract

Purpose—The purpose of this study is to develop a method to reliably characterize multiple features of the corticospinal system in a more efficient manner than typically done in transcranial magnetic stimulation (TMS) studies.

Methods—Forty TMS pulses of varying intensity were given over the first dorsal interosseous motor hot spot in 10 healthy adults. The FDI motor evoked potential (MEP) size was recorded during rest and activation to create recruitment curves. The Boltzmann sigmoidal function was fit to the data, and parameters relating to maximal MEP size, curve slope, and stimulus intensity leading to half-maximal MEP size were computed from the curve fit.

Results—Good to excellent test-retest reliability was found for all corticospinal parameters at rest and during activation with 40 TMS pulses.

Conclusions—Through the use of curve fitting, important features of the corticospinal system can be determined with fewer stimuli than typically used for the same information. Determining the recruitment curve provides a basis to understand the state of the corticospinal system and select subject-specific parameters for TMS testing quickly and without unnecessary exposure to magnetic stimulation. This method can be useful in individuals who have difficulty maintaining stillness, including children and patients with motor disorders.

Keywords

transcranial magnetic stimulation; recruitment curve; input-output curve; motor threshold

Introduction

Since its introduction in 1985, transcranial magnetic stimulation (TMS) has become an increasingly popular technique used to stimulate the human brain non-invasively and

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painlessly in awake, cooperating individuals (Barker, et al., 1985). The widespread use of TMS has led to numerous advances in motor control physiology (Hallett, 2000), and more recently in the treatment of neurological conditions (Wassermann and Zimmermann, 2012). When applied to the scalp over the primary motor cortex of the brain, the magnetic field produced by TMS induces an electric current in the cortical tissue that can activate the corticospinal tract, which consequently activates a target muscle. Surface electromyography (EMG) can then be used to monitor the motor evoked potential (MEP) in the muscle.

The recruitment curve describes the input-output properties of the corticospinal system, or how MEP size is affected by changes in TMS intensity. This relation can be affected by recruitment of cortical neurons by the TMS stimulus, the multiple parts of the corticospinal volley (direct and indirect waves), recruitment of motor neurons, and synchronization of motor neuron discharges. In the recruitment curve, there is no MEP at low intensities, a steep increase in average MEP amplitude at a particular intensity (cortical motor threshold, CMT), and then a plateau to a saturation level at higher intensities. It has been approximated using the Boltzmann sigmoidal function (Devanne, et al., 1997), which characterizes the MEP size as a function of stimulation intensity (MEP(s)) with four parameters (EMG_{base}, MEP_{sat}, s_{50}, and k) as follows:

$$MEP(s) = EMG_{base} + \frac{MEP_{sat}}{1 + e^{\frac{s_{50} - s}{k}}} \quad \text{Equation 1}$$

EMG_{base} is the baseline EMG present at rest, MEP_{sat} is the plateau value at high stimulation intensities, s_{50} is the stimulation intensity that produces a MEP half-way between EMG_{base} and MEP_{sat}, and k is the change in stimulus intensity from s_{50} that relates to a 73% change in MEP(s). The larger the value of k, the more shallow the sloping region of the curve. The MEP_{sat} value is known to result from excitation of all target motor neurons yet be smaller than the compound motor action potential resulting from electrical stimulation of peripheral nerves. This is because of desynchronization within the corticospinal tract or at the level of the spinal cord (Magistris, et al., 1998). The slope of the recruitment curve is likely related to the strength of corticospinal projections (Chen, et al., 1998). It is steeper in muscles with a lower CMT (Chen, et al., 1998), and increases with the level of tonic activity in a muscle (Devanne, et al., 1997, Hess, et al., 1987).

Good test-retest reliability of the Boltzmann curve fit, evidenced by an intra-class correlation coefficient between 0.60 and 0.77 for MEP_{sat}, s_{50}, and k, has been shown using 90 TMS stimuli (Carroll, et al., 2001) distributed over the range of stimulus intensities of the recruitment curve, and has been used to assess changes in corticospinal excitability due to certain behavioral conditions or interventions (Capaday, et al., 1999, Duclay, et al., 2011, Houdayer, et al., 2008, Thomas and Gorassini, 2005). In this paper, we explore the reliability of using fewer TMS pulses than currently used to establish the recruitment curve, and fit the Boltzmann sigmoidal function to extract the associated parameters. We hypothesize that this technique can be applied to reliably determine MEP_{sat}, s_{50}, and k for a hand muscle during rest and activation with only 40 TMS pulses. This quick and reliable method can be used to gain useful information regarding the corticospinal system while

reducing exposure to TMS, which may be particularly important when studying young individuals with difficulty remaining still and patients with motor disorders.

Methods

Participants

Ten right-handed volunteers (28 ± 8 years, 5 female) provided written informed consent to participate in this study. A history and physical examination was conducted by a neurologist and indicated that participants had no neurological impairments and were not taking medications known to influence neurological function. All experimental procedures were approved by the NIH Institutional Review Board. This study was registered with clinicaltrials.gov (NCT01019343).

Experimental Setting and Apparatus

Participants were seated comfortably facing a computer screen with their right forearm prone on a table and their left arm resting in their lap. During testing, the right shoulder was abducted approximately 30 degrees, and the right elbow was flexed approximately 90 degrees. The right index fingertip was placed on a force transducer (Strain Measurement Devices) to measure the flexion force resulting from isometric contraction of the first dorsal interosseous (FDI) muscle.

Surface EMG of the right FDI was recorded using a pair of Ag-AgCl electrodes (Natus Medical, Inc.) configured in a belly-tendon montage along the length of the muscle fibers. In order to keep electrode impedance below 20 kΩ, the skin was cleansed with alcohol and rubbed with an abrasive gel prior to placing the electrodes with conductive electrode gel. A round disc electrode was placed on the forearm proximal to the wrist and used as a reference. EMG signals were amplified and filtered (bandpass 10 – 1000 Hz, Nihon-Kohden Corp.) prior to digitizing (5000 Hz, Cambridge Electronic Design Ltd.).

Single-pulse TMS was administered through a figure-of-eight coil (Magstim Company Ltd., Magstim 200^2 monophasic stimulator, inner-loop diameter of 70mm). The coil was oriented tangentially on the scalp and pointing backward at an angle of approximately 45 degrees to induce a current in the brain flowing from the posterior to the anterior direction perpendicular to the central sulcus (Brasil-Neto, et al., 1992). The coil position used for testing (“hot spot”) was the point on the left side of the scalp eliciting MEPs in the right resting FDI of the greatest amplitude in response to a stimulus of 60% maximum stimulator output (MSO). The same coil location and orientation was used for stimulation during rest and muscle activation in the interest of time. The hot spot was marked on a tight skull cap for reference throughout testing.

Testing procedures

First, maximum voluntary isometric contractions (MVIC) of the FDI were obtained in three seven-second trials for each participant. Visual feedback of finger flexion force was provided on the computer screen. Rest breaks were given between MVIC trials as needed, and encouragement was provided during trials to achieve a maximal force. The mean force

over a five-second window of consistent force production was computed for each trial, and the peak mean force over the three trials was considered to be the MVIC force. The MVIC force was used as a reference for trials including muscle activation.

To test the repeatability of the recruitment curve in different states of muscle activation, the TMS protocol was performed twice at each of two states of muscle activation (rest, active) in four separate sessions. A description of the TMS protocol and each activation condition is provided below (see “TMS protocol”). During testing, there were two investigators working with the participant for every session; one investigator held the stimulating coil, and the other investigator operated the computer. The first two sessions and the last two sessions were performed with a different pair of investigators with an approximately 15-minute break in between to allow investigators to switch positions. When the second set of investigators began testing, they placed the TMS coil above the hot spot mark on the skull cap and then used several additional TMS pulses at 60% MSO to ensure coil placement on the hot spot. The order of the four sessions, including which pair of investigators was involved and which conditions were tested was shuffled between subjects to avoid order effects in reliability measurements (Table 1).

TMS protocol

The TMS protocol was followed in the rest and active conditions. In the rest condition, participants were facing the computer screen and relaxing the FDI muscle. In the active condition, 15% of the MVIC force was used as a target level of finger flexion. The target level was indicated as a horizontal line on the computer screen scaled to be in the middle of the visual display. Forty TMS pulses (2 sets of 20 pulses evenly distributed in 5% increments between 5% and 100% MSO) were administered. A different pseudo-random order was used for each set of 20 TMS pulses and for each participant. TMS stimulus intensities were programmed and controlled through software (Signal, Cambridge Electronic Design Ltd.)

Each trial within each session began with a beep, included a single TMS pulse at 2.5 seconds, and then a rest period for 7.5 seconds, thereby maintaining an inter-stimulus interval of 10 seconds. In the active condition, the beep cued the beginning of isometric finger flexion. Sufficient practice was given for participants to consistently achieve the target force, as seen through visual feedback on the computer screen, before the TMS pulse was given.

The mean un-rectified EMG over each trial was first subtracted from the EMG signal of that trial in order to correct for any offset. The peak-to-peak amplitude of the un-rectified FDI EMG signal was computed in the 50ms response window beginning 10ms after the TMS pulse, and will be referred to as the peak-to-peak response.

Curve fitting

Baseline EMG (EMG_{base}) was computed as the mean peak-to-peak response over all trials with stimulus intensities at or below 20% MSO. EMG_{base} was used in Equation 1, and the remaining three parameters (MEP_{sat}, s_{50}, k) were determined from the curve fit. Curve

fitting of the Boltzmann function (Equation 1) was done using the Levenberg-Marquardt algorithm (Seber and Wild, 2003) in Matlab (nlinfit function, The MathWorks, Inc.).

Outcome measures

The three primary outcome measures are the three recruitment curve parameters (MEP_{sat}, s_{50}, k). Mean normalized pre-stimulus EMG activity (during the 100ms period before the TMS pulse) was computed as a secondary outcome measure to check for consistent muscle activation during all trials in the active condition. EMG signals were rectified prior to averaging. Normalization was accomplished by dividing by the mean rectified EMG during that subject's MVIC trial with the peak mean force.

Statistical Analysis

Pearson correlation coefficients were used to assess the relationship between the three primary outcome measures.

The mean difference and the 95% Bland-Altman limits of agreement (Bland and Altman, 1986) were computed to assess bias and variability in recruitment curve parameters between the test and retest sessions.

The intraclass correlation (ICC) method (Shrout and Fleiss, 1979) was used to examine test-retest reliability of the recruitment curve parameters (MEP_{sat}, s_{50}, k). ICC(2,1), which is concerned with repeatability of the absolute value of the outcomes, was computed. Data from repeated testing sessions were used for this analysis. For example, in Subject 1 at rest, sessions 1 and 4 were used to assess reliability of recruitment curve parameters (see Table 1).

In the interest of time in a TMS experiment, it would be convenient if the input-output properties of the corticospinal system could be determined reliably with less than 40 pulses. Since the TMS protocol consisted of two sets of 20 evenly distributed pulses, the recruitment curve parameters were derived from each set of 20 pulses in a secondary analysis. ICC(2,1) was computed to assess the reliability of outcomes between the first sets of each curve protocol and between the second sets of each curve protocol (two test-retest comparisons) for each muscle activation condition.

The assumption of a Gaussian distribution was evaluated by the Shapiro-Wilk test. An alpha level of 0.05 was used. Statistical analyses were performed using SAS version 9.2 and SPSS version 21.

Results

General results

All participants were able to complete testing as expected in a single visit with no adverse events. All subjects were able to rest completely during the rest trials. During the active condition, a consistent level of contraction was achieved within each subject across the two sessions ($p = 0.21$).

Figure 1 provides an example of the Boltzmann curve fit to data from the TMS protocol in one representative subject (Subject 10, Sessions 2 and 3). Over all sessions in all subjects, the Boltzmann function provided a good fit in the rest condition (mean ± SD coefficient of determination, $R^2 = 0.85 \pm 0.09$), and in the active condition ($R^2 = 0.96 \pm 0.03$). The baseline EMG is larger, the stimulus intensity in the neighborhood of the CMT is lower, the sloping part of the curve is steeper, and the maximum MEP value is larger in the active condition compared to the rest condition.

Recruitment curve parameters (MEP_{sat}, s_{50}, and k) were mostly uncorrelated in the rest and active conditions. The only significant correlation amongst them was between s_{50} and k during muscle activation (Pearson's correlation coefficient, r = 0.74).

Recruitment curve parameters

Bland–Altman plots are shown in Figure 2 for MEP_{sat} (a), s_{50} (b), and k (c) in the rest (left panel) and active (right panel) conditions. In these plots, the mean test-retest difference for each parameter is plotted against the mean value of the parameter. In both conditions, the negative bias in the mean MEP_{sat} difference (dotted line) indicates that MEP_{sat} was estimated to be slightly larger in the second compared to the first assessment, however this difference was not statistically significant using a paired samples t-test ($t(9) = 0.276$, $p = 0.789$). s_{50} and k values are evenly distributed around a near-zero difference in the rest and active conditions. The 95% limits of agreement (solid lines) for MEP_{sat} are similar between conditions. However, they are smaller, indicating less variability, in the active versus rest condition for s_{50}, and k.

Table 2 presents mean test and retest values, ICC(2,1), and the 95% confidence limits of ICC(2,1) for recruitment curve parameters (MEP_{sat}, s_{50}, and k) in the rest and active conditions. According to published recommendations (Fleiss, 1986), ICC values above 0.75 represent excellent reliability, values between 0.4 and 0.75 represent fair to good reliability, while values below 0.4 represent poor reliability. In both conditions, the three parameters reach good or excellent reliability, although the k parameter at rest is highly variable.

Table 3 shows ICC(2,1) for recruitment curve parameters determined using 20 TMS pulses in the rest and active conditions. The test-retest comparison denoted by Set 1 is between the first sets of each TMS protocol, and Set 2 is the comparison between the second sets of each curve protocol. The repeatability of parameters using only 20 TMS pulses (Table 3) is poorer and the confidence intervals are larger than the same measures made with 40 TMS pulses (Table 2). In addition, there is a high degree of variability in ICC(2,1) between Set 1 and Set 2 in both conditions.

Discussion

In this study, we demonstrated that curve fitting with the Boltzmann sigmoidal function can be used to reliably assess MEP_{sat}, s_{50}, and k of the FDI using 40 TMS pulses. Given the ease with which the Boltzmann curve and its associated parameters can be reliably determined, we suggest the use of the curve parameters as a basis for assessing changes in cortical excitability.

Recruitment curve

During muscle contraction, corticospinal drive to the muscle is specified as that needed to achieve the target force, whereas it is unspecified and possibly fluctuating when the muscle is at rest since there is a broad range of drive that is all subthreshold (from just subthreshold to inhibition). This is evidenced by the greater reliability (Table 2) of the recruitment curve parameters in the active condition than the rest condition. Voluntary muscle activation is also known to increase MEP size (Devanne, et al., 1997, Hess, et al., 1987), and the steepness of the recruitment curve (Devanne, et al., 1997). This could be seen by the larger MEP_{sat}, and the smaller k (the inverse of k is related to the slope, so a lower value indicates a steeper relation) when the muscle was active compared to resting (Table 2).

The three recruitment curve parameters represent different aspects of the corticospinal system; however, they can be related. The correlation between k and s_{50} during muscle activation is likely due to the fact that the s_{50} parameter occurs in the middle of the sloping region of the recruitment curve. Therefore a change in the slope of the curve can lead to a change in the s_{50} intensity. This effect is only present during muscle activation because a decrease in k (increase in steepness) will co-occur with a decrease in s_{50} more so in a steep curve (active condition) than a shallow one (rest condition).

The test-retest reliability of the parameters of the Boltzmann curve fit of the FDI MEP at rest with 40 points between two sessions on one day was similar to previously published results using 90 points between three sessions on different days (Carroll, et al., 2001). Carroll and colleagues found MEP_{sat}, s_{50}, and k to have ICC(2,1) values of 0.60, 0.63, and 0.77, respectively, indicating that MEP_{sat} was the least reliable and k was the most reliable parameter. Interestingly, the ICC(2,1) of MEP_{sat}, s_{50}, and k in this study were 0.94, 0.84, and 0.60, respectively, demonstrating that MEP_{sat} was the most reliable, and k was the least reliable parameter of the three. This discrepancy may be due to the different distributions of stimulus intensities used in the studies. Carroll and colleagues did not test as many high stimulus intensities, which are required to estimate MEP_{sat}, as was done in this study. Similarly, Carroll and colleagues sampled the stimulus intensities near the sloping region of the curve, necessary to estimate k, more than in this study. The divergent results between the two studies do not discredit the curve fitting method in general since the absolute reliability of all three parameters in both studies were good to excellent. Instead, these results suggest that when fitting the Boltzmann curve to experimental data, additional TMS pulses can be used in particular regions of interest in order to improve specific parameter estimates.

For the parameter k in the rest condition, the large confidence limits of ICC(2,1), and limits of agreement on Figure 2c are likely due to one subject who had an especially large increase in k from the test to the retest. This data point can be seen on the bottom right part of Figure 2c in the rest condition. Although the variability is more consistent between conditions for MEP_{sat} and s_{50} compared to k, it is important to consider the consequences of this variability in experimental studies. Use of the methods presented in this study to test changes in cortical excitability must be limited to cases where the expected changes are larger than the inherent variability in the method.

The approximately 0.5mV increase in MEP_{sat} estimates from the test to the retest in both muscle activation conditions (non-zero mean difference in Figure 2a) raises the possibility that there was a slight increase in cortical excitability due to the 0.1Hz stimulation during testing. However, a previous study of 0.1 Hz TMS of the motor cortex at 5%MSO above resting CMT showed no change in cortical excitability (Chen, et al., 1997). This discrepancy could be due to differences in the stimulation intensity between studies and highlights the benefit of obtaining the full recruitment curve rather than sampling at only one stimulation intensity. Nonetheless, the mean difference in repeated MEP_{sat} estimates was small and all parameter measurements were repeatable, so any changes in cortical excitability that may have occurred do not impact the interpretation of these results.

There are several limitations of the curve fitting method presented in this study. It is known that input-output properties of the corticospinal system for different muscles vary (Malcolm, et al., 2006), and it may thus require a different number of TMS pulses to determine the recruitment curve parameters. In addition, this method would not be appropriate for muscles with saturation levels that are not measurable within the range of stimulator output available from the particular TMS stimulator used. In this case, there would not be sufficient data to fit the sigmoidal curve and estimate the parameters. Finally, a least-squares fit of EMG responses to TMS was used in this study. However, a recent study has suggested that this may underestimate the slope of the recruitment curve because of differences in the distribution of MEP variability at different stimulus intensities (Goetz and Peterchev, 2012). This may be partially rectified by logarithm-normalizing the MEP values prior to curve fitting (Nielsen, 1996), but that was not done in this study. Future studies are required to compare results from the method presented here and alternative methods that account for the variability of MEP values.

Application to TMS studies

Results from this study can be applied directly to TMS experiments with only minor modifications. Some software packages for EMG analysis provide the ability to fit a Boltzmann sigmoidal curve to experimental data (for example, Signal software, Cambridge Electronic Design Ltd.). A benefit of this TMS protocol is that the investigators do not require online viewing of the MEP, which allows full focus to be on the coil and maintenance of the coil position.

In general, when assessing changes in the corticospinal system due to particular experimental conditions, the parameters of the recruitment curve can be used as the outcome measures. This would provide a more complete analysis of the state of the corticospinal system than the method often used of comparing MEP size between experimental conditions at the stimulus intensity required for a 1mV MEP at rest (Rosenkranz and Rothwell, 2003). The acquisition of a 1mV MEP may not always be possible in all muscles and in certain subject groups, including children (Garvey and Gilbert, 2004). If it is not feasible to obtain the full recruitment curve a second time to assess changes due to an intervention, the s_{50} value could be used as a test stimulus intensity, rather than the 1mV stimulus. The s_{50} value provides a subject-specific intensity that is between the baseline EMG value and the MEP saturation value allowing for the measurement of both inhibition and facilitation of the

MEP. Indeed, the 1mV method is irrational since 1mV will correspond to differing points on the recruitment curve for different subjects. If 1mV is near a subject's MEP_{sat}, then little facilitation will be possible. Similarly, if 1mV is near EMG_{base}, then little inhibition will be possible.

The methods described here provide the MEP recruitment curve reliably for fewer stimuli than typically used. This savings in time can be useful when the subsequent use of longer and/or more intense TMS protocols, including paired pulse TMS and repetitive TMS, are planned. Shorter and more efficient TMS protocols can be especially beneficial in high risk groups, including children.

It is clear from Table 3 that using fewer than 40 TMS pulses has a negative effect on the reliability of the recruitment curve parameters. Although it is certainly possible to fit a sigmoidal function to any set of data, it is not advisable to do so with fewer than 40 TMS pulses as insufficient sampling of the recruitment curve can lead to unreliable and highly variable measurements.

There are variations of the methods presented here that may be preferable in certain situations. For example, EMG_{base} is computed as the mean peak-to-peak response over all trials with stimulus intensities at or below 20% MSO. However, this definition would not suit the analysis of a muscle with CMT lower than 20% MSO since EMG_{base} would be biased by small amplitude MEP values. In this case, it may be useful to compute the mean EMG value in a given pre-stimulus time interval for each of the 40 TMS trials, and define EMG_{base} as the mean over the 40 trials. Another variation can include estimating MEP_{sat} from the raw data or the recruitment curve parameters, and then fitting the Boltzmann sigmoidal function with only two free parameters (s_{50} and k). If the estimate of MEP_{sat} is determined using the recruitment curve parameters, the process can be iterated until there is sufficient stability in the parameters. In this study, the Boltzmann sigmoidal curve is used to describe the recruitment curve. There are also different types of sigmoid curves that can be used, including a cumulative Gaussian sigmoid curve (Peterchev, et al., 2013) and a Hill-type function (Goetz and Peterchev, 2012), for which test-retest reliability has not yet been shown.

The CMT can be appreciated visually on the recruitment curve; however there is no particular parameter of the sigmoidal curve fitting procedure that represents this important neurophysiologic feature. Excellent reliability of one quantitative method to estimate CMT from a sigmoidal curve fit to experimental data has been shown previously (Carroll, et al., 2001). In this method, CMT is defined as the stimulus intensity where the line tangent to the recruitment curve at the s_{50} point intersects EMG_{base}. Benefits of using this method include the ability to estimate CMT without additional TMS pulses, and that it is based on the curve and not on an arbitrary voltage level (for example, 0.05 mV for a resting muscle) as most other CMT estimation methods are. The drawback of quantitative methods to estimate CMT from the recruitment curve is that they may magnify any variability in the curve fit and lead to less reliable estimates. Of the many CMT estimation procedures based on an arbitrary EMG voltage level, and summarized by the International Federation of Clinical Neurophysiology (IFCN) (Groppa, et al., 2012), “adaptive threshold hunting” uses the

fewest stimuli (Awiszus, 2003). In consideration of testing time and exposure to TMS, we suggest that it may be favorable to first determine the recruitment curve as described in this study, and then use the data points already collected as an input of prior information to the threshold hunting algorithm mentioned above. This process can yield a mathematically valid CMT estimate with only 14 additional stimuli (Awiszus, 2011), which is far fewer than what is used by most other CMT estimation methods.

In this study, the use of 60 %MSO as a TMS intensity for hot spot mapping may have been too near MEP_{sat} to allow unique characterization of the hot spot since stimulation of multiple scalp regions at high intensities may evoke similar EMG responses. Consequently, use of a sub-maximal (and also supra-threshold) TMS stimulus in future studies may be important for improved hot spot localization, which would likely result in increased reliability of recruitment curve parameters. This study presents test-retest reliability within one day, and therefore caution should be used when relating these results to tests between days. In addition, it should be noted that this reliability study does not address validity, which must be demonstrated in future studies.

In summary, we have shown good to excellent test-retest reliability of a curve fitting method to extract parameters related to corticospinal excitability using only 40 TMS pulses distributed over the full range of stimulator output. This method can be particularly useful in individuals with difficulty maintaining stillness, such as children and patients with motor disorders. The s_{50} parameter provides an ideal test stimulus intensity for assessing changes in cortical excitability as it produces a half-maximal MEP that can be manipulated experimentally to either show facilitation (an increase in MEP) or inhibition (a decrease in MEP).

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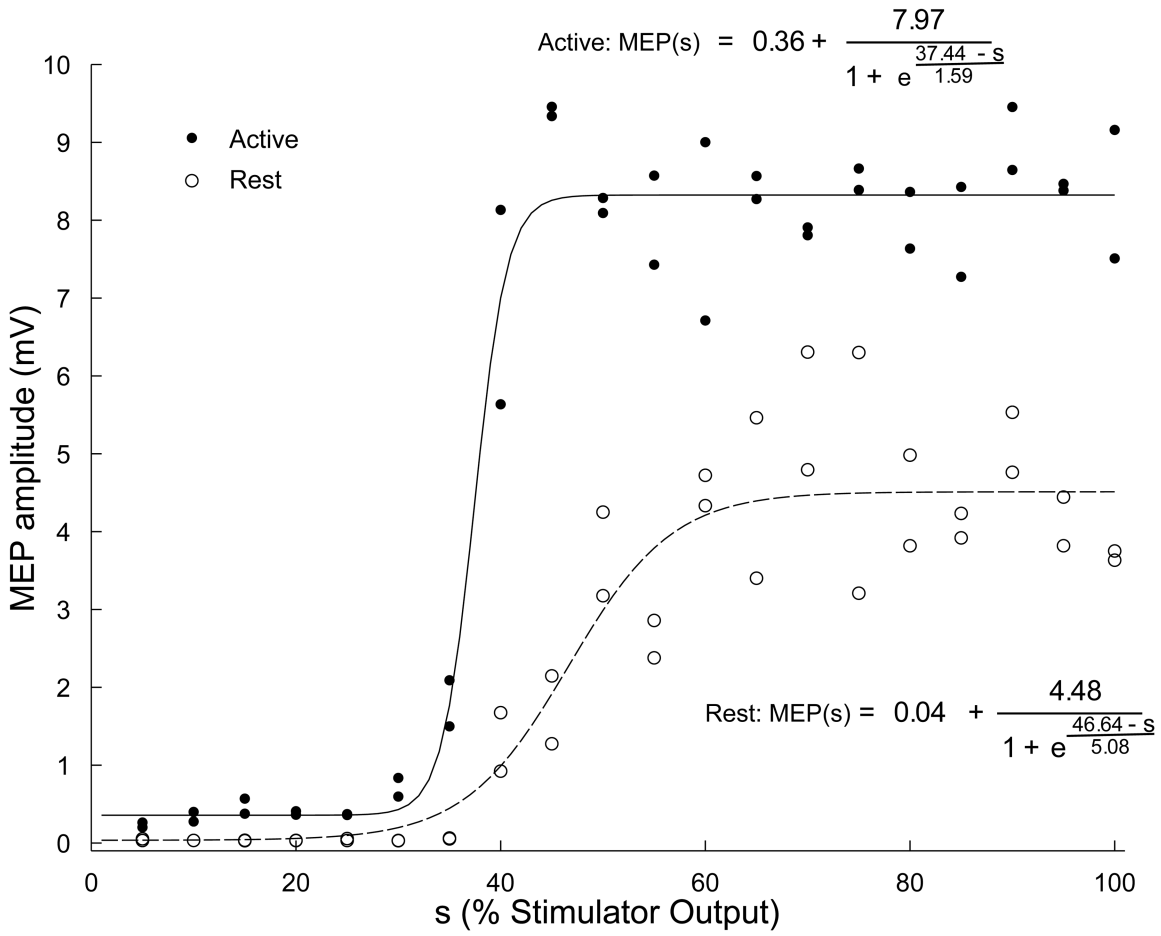

Figure 1.

Boltzmann curve fit to data from the curve protocol in one representative subject (Subject 10, Sessions 2 and 3). $R^2 = 0.89$ for the rest condition (dashed line), and $R^2 = 0.97$ for the active condition (solid line). The baseline EMG is larger, the stimulus intensity in the neighborhood of the cortical motor threshold is lower, the sloping part of the curve is steeper, and the maximum MEP value is larger in the active condition compared to the rest condition. The equation of the curve for each condition is given on the figure.

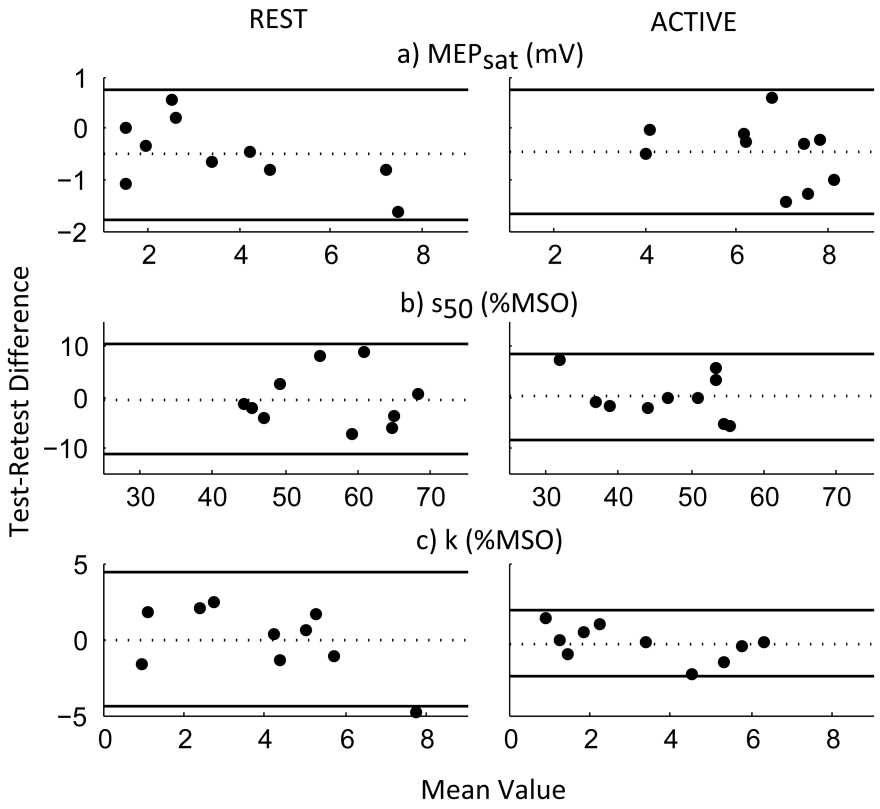

Figure 2.

Bland-Altman plot for MEP_{sat} (a), s_{50} (b), and k (c) in the rest (left panel) and active (right panel) conditions. Each point represents the test-retest difference for one subject. The dashed lines represent the mean difference over all subjects, and the solid lines represent the 95% limits of agreement (mean difference ± 1.96*standard deviation).

Table 1

Investigator pair (A,B) and activation condition (Rest, Active) for each session. For example, investigator pair A tested Subject 6 in the Active condition during the third session.

Subject	Session Number			
	1	2	3	4
	Rest	Active	Active	Rest
1	A	A	B	B
2	A	A	B	B
3	A	A	B	B
4	A	A	B	B
5	A	A	B	B
6	B	B	A	A
7	A	A	B	B
8	B	B	A	A
9	B	B	A	A
10	B	B	A	A

Table 2

Test and retest recruitment curve parameter values, ICC(2,1), and the 95% confidence limits (95% CL) of ICC(2,1) from the dataset with 40 TMS pulses in the rest and active conditions.

Parameter	Condition	Test (M ± SD)	Retest (M ± SD)	ICC(2,1)	95% CL of ICC(2,1)
MEP_{sat} (mV)	Rest	3.46 ± 2.02	3.96 ± 2.41	0.94	(0.67, 0.99)
	Active	6.31 ± 1.38	6.77 ± 1.58	0.88	(0.49, 0.97)
s_{50} (%MSO)	Rest	56.7 ± 9.2	56.1 ± 9.4	0.84	(0.46, 0.96)
	Active	46.7 ± 7.9	46.6 ± 9.3	0.87	(0.57, 0.97)
K (%MSO)	Rest	3.9 ± 1.8	4.0 ± 2.9	0.60	(-0.05, 0.88)
	Active	3.2 ± 1.8	3.4 ± 2.4	0.89	(0.60, 0.97)

Table 3

ICC(2,1), and the 95% confidence limits (95% CL) of ICC(2,1) from Set 1 and Set 2 with 20 TMS pulses in the rest and active conditions.

		Set 1		Set 2	
Parameter	**Condition**	**ICC(2,1)**	**95% CL of ICC(2,1)**	**ICC(2,1)**	**95% CL of ICC(2,1)**
MEP_{sat} (mV)	Rest	0.91	(0.71, 0.98)	0.89	(0.41, 0.98)
	Active	0.63	(0.14, 0.91)	0.91	(0.70, 0.98)
s_{50} (%MSO)	Rest	0.83	(0.49, 0.96)	0.59	(-0.06, 0.88)
	Active	0.77	(0.32, 0.94)	0.91	(0.68, 0.98)
K (%MSO)	Rest	0.42	(-0.19, 0.81)	0.22	(-0.53, 0.74)
	Active	0.72	(0.19, 0.92)	0.56	(-0.10, 0.87)