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Research paper

# Fast acquisition of resting motor threshold with a stimulus–response curve – Possibility or hazard for transcranial magnetic stimulation applications?

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ABSTRACT

*Objective:* Previous research has suggested that transcranial magnetic stimulation (TMS) related cortical excitability measures could be estimated quickly using stimulus–response curves with short interstimulus intervals (ISIs). Here we evaluated the resting motor threshold (rMT) estimated with these curves.
*Methods:* Stimulus-response curves were measured with three ISIs: 1.2–2 s, 2–3 s, and 3–4 s. Each curve was formed with 108 stimuli using stimulation intensities ranging from 0.75 to 1.25 times the $rMT_{guess}$, which was estimated based on motor evoked potential (MEP) amplitudes of three scout responses.
*Results:* The ISI did not affect the rMT estimated from the curves ($F = 0.235$, $p = 0.683$) or single-trial MEP amplitudes at the group level ($F = 0.90$, $p = 0.405$), but a significant subject by ISI interaction ($F = 3.64$; $p < 0.001$) was detected in MEP amplitudes. No trend was observed which ISI was most excitable, as it varied between subjects.
*Conclusions:* At the group level, the stimulus–response curves are unaffected by the short ISI. At the individual level, these curves are highly affected by the ISI.
*Significance:* Estimating rMT using stimulus–response curves with short ISIs impacts the rMT estimate and should be avoided in clinical and research TMS applications.


## 1. Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation method (Barker et al., 1985; Ilmoniemi et al., 1999) widely used in research and clinical applications (Rossini et al., 2015; Lefaucheur et al., 2020). Regardless of the exact application, one of the most influential parameters in all TMS applications is the strength at which the stimulation is applied (Pretalli et al., 2012; Rossini et al., 2015). A too low stimulation intensity (SI) may not activate the target area. In contrast, an excessively high SI may activate the target and the neighboring regions, resulting in a loss of focality (Kallioniemi and Julkunen, 2016; Konakanchi et al., 2020). Also, high SI may decrease the safety of the stimulation (Rossi et al., 2009). A constant SI across the subjects may not induce similar effects in different individuals because the level of cortical excitability varies substantially between subjects (Säisänen et al., 2008; Sollmann et al., 2017). To tackle this, the conventional approach is to normalize the SI to the subject-specific cortical excitability. This is done by first estimating the resting motor threshold (rMT) and, after that applying some sub- or supra-threshold percentage of rMT (Rossini et al., 2015). The rMT is defined as the minimal SI needed to induce a motor evoked potential (MEP) in a relaxed target muscle in 50% of the stimulations (Rossini et al., 2015). The rMT can be estimated with various manual methods, such as Rossini-Rothwell (Rossini et al., 2015)

*Abbreviations:* APB, abductor pollicis brevis; EMG, electromyography; ISI, interstimulus interval; MEP, motor evoked potential; MRI, magnetic resonance imaging; MSO, maximum stimulator output; rMT, resting motor threshold; $rMT_{estimate}$, resting motor threshold estimated with stimulus–response curves; $rMT_{guess}$, resting motor threshold estimated with prior information and three scout pulses; $rMT_{RR}$, resting motor threshold estimated with the Rossini-Rothwell method; $rMT_{threshold}$, resting motor threshold estimated with the threshold-hunting method; $rMT_{true}$, true resting motor threshold in simulations; SI, stimulation intensity; TMS, transcranial magnetic stimulation.

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and Mills-Nithi (Mills and Nithi, 1997), and semi-automatic procedures, such as threshold-hunting (Awiszus, 2003), that all lead to similar results (Tranulis et al., 2006).

From a physiological perspective, the rMT reflects the excitability of the motor pathways (Rossini et al., 2015) and is influenced by central and peripheral motor systems (Weber and Eisen, 2002; Rossini et al., 2015). By definition, the rMT is not meant to evoke an MEP at each stimulation, and commonly, successive MEPs are highly variable in amplitude (Kiers et al., 1993). However, the rMT is very stable if no substantial brain state change has occurred (Kimiskidis et al., 2004; Danner et al., 2008; Sankarasubramanian et al., 2015; Engelhardt et al., 2019; Ter Braack et al., 2019).

The time needed to estimate the rMT depends on the method and is generally in the range of several minutes. The time associated with estimating the rMT with manual methods may be longer than that of semi-automatic, as in manual methods, the user needs to guess and test potential rMT intensities. In contrast, in semi-automatic methods, previous data is applied to help narrow the search. Some investigators have attempted to decrease the time needed to acquire the rMT (Awiszus, 2011; Qi et al., 2011). The shortening of the estimation time could be achieved by using subject- or group-specific priors, decreasing the number of pulses or the time between two pulses, i.e., the interstimulus interval (ISI). However, the ISI cannot be absurdly short, as ISIs of 1–4 s or less may cause cumulative effects to the MEPs (Möller et al., 2009; Julkunen et al., 2012; Rossini et al., 2015; Pellicciari et al., 2016). Thus, the general rule of thumb is to use an ISI of at least 5 s. However, the exact neurophysiological phenomenon underlying the 5 s rule remains unknown but could be associated with the time it takes a neuron to recover from a TMS pulse. The 5 s ISI results (Julkunen et al., 2012; Rossini et al., 2015; Pellicciari et al., 2016) have been obtained by keeping the SI constant. It is unknown whether similar effects would be received with varying SIs used to estimate the rMT. A study by Mathias et al. (2014) investigated the stimulus–response curves, in which ISIs ranging from 1.4 s up to 4 s were applied (Mathias et al., 2014). Stimulus-response curves, also called input–output, recruitment, or threshold curves, utilize a range of SIs varying from sub- to supra-rMT and reflect a wide range of cortical excitability (Möller et al., 2009; Julkunen et al., 2011; Kukke et al., 2014; Kallioniemi et al., 2015a). The different SIs are applied in a random order, and a Boltzmann-like model is fitted to the data. The stimulus–response curves are sigmoidally shaped with a steep slope around 100% of rMT before the MEP amplitudes plateau. The study by Mathias et al. found no differences between stimulus–response curves obtained with different short ISIs. This finding suggests that although a short ISI affects the MEPs evoked with constant SI, the cumulative effects might be avoided when using varying SIs given in random order.

Previously, we evaluated the effects of repetition on MEP amplitudes induced with a constant intensity (Pitkänen et al., 2017). Altogether, 120 successive pulses with an ISI of 1 s were given, and trends in individual MEP amplitudes were evaluated. Instead of systematic effects on the consecutive MEPs, each individual responded to the sequence differently, also suggested by other studies (Touge et al., 2001; Romero et al., 2002; Strigaro et al., 2016). Therefore, we hypothesized that even though the stimulus–response curves may not show variation at group level, these curves include considerable between-subjects variation. Hence, our main aim was to study the within-subject reproducibility of rMT obtained with stimulus–response curves using short ISIs. To investigate this, we measured stimulus–response curves with similar ISIs as Mathias et al. To form the curves, we used prior group level information from our previous study (Julkunen et al., 2011) together with a short initial measurement.

## 2. Methods

### 2.1. Subjects

The study included thirteen healthy right-handed volunteers (9 females, 4 males, age range: 22–60 years) without any history of neurological or psychiatric disorders. The study was approved by the local ethics committee (ethical permission 8/2012), and written informed consent was collected from all the participants. The work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. During the measurements, the subjects were not under the influence of alcohol, any drug, or pharmaceutical impacting the function of the central nervous system or cortical excitability.

### 2.2. Experiments

Before starting the TMS measurements, the participants underwent a magnetic resonance imaging (MRI) session with a 3 T MRI device (Philips Achieva 3.0 T, TX, Philips, Eindhoven, The Netherlands). The MR images were used to perform neuronavigated TMS with an eXimia system (version 3.2.2, Nexstim Plc., Helsinki, Finland). Biphasic single-pulses were administered with a figure-of-eight coil. Electromyography (EMG) was measured from the right-hand abductor pollicis brevis (APB) muscle with Ag-AgCl electrodes in a belly-to-tendon montage with an integrated and stimulus-locked EMG device. The recorded EMG signal was sampled at 3 kHz with a resolution of 0.3 μV and voltage range from −7.5 mV to +7.5 mV.

The examination began by mapping the left cortical representation for the APB muscle according to current recommendations (Rossini et al., 2015) by holding the coil so that the induced electric field was perpendicular to the nearest sulcus. Mapping was conducted with an SI that produced MEPs with an amplitude of 1–2 mV. At this intensity level, the variability of MEP amplitudes decreases, facilitating finding the optimal target location for the stimulation, i.e., the hotspot (Rossini et al., 2015). At the location which consistently evoked the highest amplitude MEPs for APB, the coil was rotated within ±90° to find the optimal electric field direction. This optimal location and direction were defined as the APB target. The number of stimuli applied to determine the target location varied between subjects (range 39–100), as finding the target location is a unique process for each individual and is influenced by how easy the target is to find. Three single pulses were given at the target with an ISI of at least 5 s and an intensity producing MEPs between 1 and 2 mV. The peak-to-peak amplitudes of these three pulses were used to estimate the rMT ($rMT_{guess}$) according to Equation (1), which was derived based on the data from our previous study (Julkunen et al., 2011).

$$rMT_{guess} = \frac{SI + 8.83e^{-3} * (A - 50\mu V)}{1 + 0.363e^{-3} * (A - 50\mu V)} \quad (1)$$

In this equation, $A$ denotes the median amplitude of the three repeated single pulses in microvolts and $SI$ the stimulation intensity.

After defining the $rMT_{guess}$, three single-pulse TMS sequences were applied at the target with a varying ISI: 1.2–2 s, 2–3 s, and 3–4 s. ISIs were jittered to avoid any interference of habituation (Pitkänen et al., 2017) and expectation effects. These sequences were given in a randomized order by repeating a train of nine SIs twelve times. Each train included SIs of 0.75 to 1.25 times of $rMT_{guess}$ at 0.05 intervals, and the trains were repeated continuously. Thus, in total, 108 pulses were given per sequence. After that, for comparison purposes, the rMT was estimated separately

with the Rossini-Rothwell method and the threshold-hunting paradigm using an ISI of at least 5 s. In the Rossini-Rothwell approach, the minimum intensity evoking 5/10 responses was set as the rMT ($rMT_{RR}$), whereas the threshold-hunting paradigm determined the rMT with 20 pulses ($rMT_{threshold}$).

### 2.3. Data analysis

MEPs with a peak-to-peak amplitude of at least 50 μV and without visibly observed pre-stimulus muscle contraction for 2 s before the pulse were accepted as responses. The MEPs from the three ISI sequences were analyzed offline in an eXimia workstation (version 3.2.2) by verifying the automatically set MEP amplitudes. The resting stimulus–response curves for each ISI were assessed using a previously published method employing maximum likelihood estimation of the threshold (Awiszus, 2003; Julkunen et al., 2011). The stimulus–response curves were characterized based on slope (% maximum stimulator output, MSO/μV), relative slope (% $rMT_{estimate}$/μV), $rMT_{estimate}$(% MSO), spread (% MSO), and relative spread (% $rMT_{estimate}$).

In addition to purely evaluating the stimulus–response curves with experimental data, we also performed simulations to assess the number of stimuli required to estimate the rMT. These were done using Monte Carlo simulations (10000 repeats at "true" rMTs ($rMT_{true}$) between 25 and 65%-MSO at 1%-MSO intervals). The simulations were conducted on the pre-defined SIs as experimentally described. The $rMT_{guess}$ was estimated in the simulations based on the experimentally observed difference between the $rMT_{guess}$ and $rMT_{threshold}$, for which the distribution was determined using the bootstrapping mean of the difference 100,000 times to determine the mean and standard deviation. These values were used in generating the $rMT_{guess}$ value for the Monte Carlo simulations by randomizing the aforementioned difference using Matlab-function normrnd. The SIs used in the simulations were then determined based on that $rMT_{guess}$ value. Then, the order of the 11 SIs was randomized within 15 epochs resulting in a total of 165 trials simulated per estimated rMT value. The occurrence of response was simulated with a cumulative distribution function (Awiszus, 2003; Julkunen, 2019) constructed based on $rMT_{true}$ and the assumed spread of 0.07*$rMT_{true}$ (Awiszus, 2003). After each stimulus, an estimate of the rMT was determined based on the probability density function applied in (Awiszus, 2003) to seek the most likely threshold corresponding with $p = 0.5$ in the cumulative distribution function. After each simulation, the relative error between the $rMT_{true}$ and the estimated rMT was evaluated. From all simulations, the 95% percentile was calculated for the relative error at each $rMT_{true}$ to evaluate the paradigm and its ability to reach satisfactory confidence at a theoretical level.

### 2.4. Statistics

At the group level, the effects of ISI on stimulus–response curve-related parameters were assessed with a repeated-measures ANOVA. If Mauchly's test of sphericity was violated, Greenhouse-Geisser correction was applied. For post-hoc analysis, Bonferroni correction was used. At the individual level, differences between MEP amplitude distributions were evaluated with a non-parametric Friedman ANOVA. The statistical significance level was set at $p < 0.05$, and the analyses were performed in SPSS 22 (IBM Corporation, Somers, NY, USA).

## 3. Results

The participants tolerated the protocols well, and no complications were observed with any of the ISIs. In one participant, the $rMT_{guess}$ was miscalculated (ID 7), and in another subject (ID13), the stimulus–response characteristics were highly abnormal and against previous literature (Möller et al., 2009; Julkunen et al., 2011; Mathias et al., 2014), see Appendix Fig. A1. These participants were excluded from the analyses, and thus, altogether, 11 participants were included.

In the stimulus–response curves, there was a main effect of subject, as subjects showed different MEP amplitudes at comparable SIs ($F(4,86) = 32.55$, $p < 0.001$). At the group level, the ISI did not affect the $rMT_{estimate}$ ($F(1.22) = 0.24$, $p = 0.683$) or single-trial MEP amplitudes ($F(1.93) = 0.90$, $p = 0.405$), but a significant subject by ISI interaction ($F(8.65) = 3.64$; $p < 0.001$) was observed in MEP amplitudes (Figs. 1 and 2). The MEP amplitude distributions were impacted by ISI in 3 subjects ($p < 0.05$, Fig. 2). No clear trends on the excitability order in the stimulus–response curves measured with different ISIs were observed. Instead, subjects reacted to the ISIs quite differently (Fig. 3). ISI did not affect slope ($F(2) = 0.02$, $p = 0.980$), relative slope ($F(2) = 0.01$, $p = 0.994$), spread ($F(2) = 1.11$, $p = 0.349$) or relative spread ($F(1.33) = 0.99$, $p = 0.363$) at the group level.

rMTs estimated with different methods, i.e., stimulus–response curve ($rMT_{estimate}$), $rMT_{guess}$, $rMT_{RR}$, and $rMT_{threshold}$, differed at the group level ($F(2.43) = 4.14$, $p = 0.022$). In the Bonferroni post-hoc analysis, no differences between any two rMT estimates were found ($p > 0.05$). At the individual level, the rMTs, however, varied (Table 1).

The Monte Carlo simulations demonstrated that the concept of using stimulus–response curves in the estimation of rMTs would be feasible theoretically (Fig. 4). Relative error observed between the estimated and $rMT_{true}$ dropped below 5% before the 4th trial was finalized. This indicates that theoretically, <44 stimuli were needed without accounting for the between-subjects effects observed in the experiments at short ISIs. Due to the different impacts of ISI in subjects, however, with real data, substantially >100 stimuli would be required to reach a reliable rMT estimate regardless of the applied ISI.

## 4. Discussion

In this study, we evaluated the within-subject variability in stimulus–response curves with short ISIs. This was done to test the possibility of reducing the time in assessing motor cortical excitability, i.e., rMT, which can be obtained readily with these

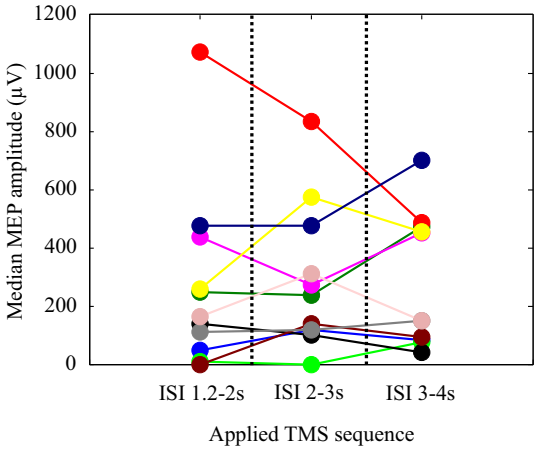


**Fig. 1.** Median motor evoked potential (MEP) amplitudes at different interstimulus interval (ISI) stimulus–response curves. The different colors represent different subjects. In some subjects, the median MEP amplitude decreases with increasing ISI, whereas, in some subjects, the opposite occurs. In the rest, the median amplitude does not change linearly with ISI.

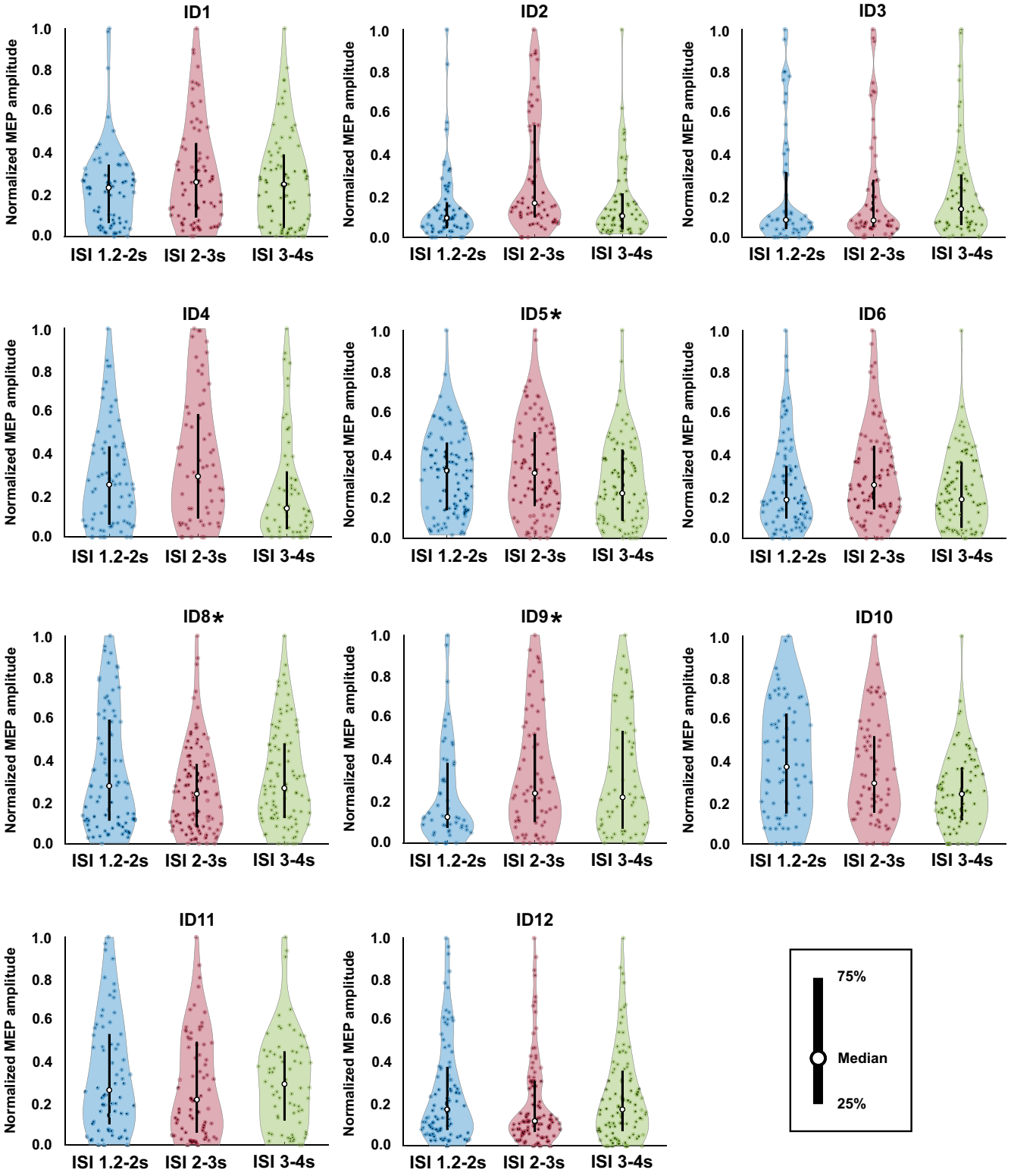


**Fig. 2.** Motor evoked potential (MEP) distributions at each interstimulus interval (ISI) normalized to the maximum amplitude of the stimulus–response curve. Wider sections reflect higher probability, and skinnier sections lower probability. No clear trends in distributions can be seen; however, the MEP amplitude distributions differed significantly only in 3 subjects (ID5, ID8, ID9). Statistics are from the non-parametric Friedman Test. Significant ($p < 0.05$) within-subject differences are marked with an asterisk after the ID number.

curves. Our main finding was that even though at the group level the estimated rMTs did not differ between the ISIs, at the individual level, the subjects reacted differently to each ISI without consistency.

One possible explanation for the varying stimulus–response curve behavior is that subjects differed in time when the neurons "recovered" from the previous pulse, as suggested by our previous study (Pitkänen et al., 2017). This is especially plausible as the SIs

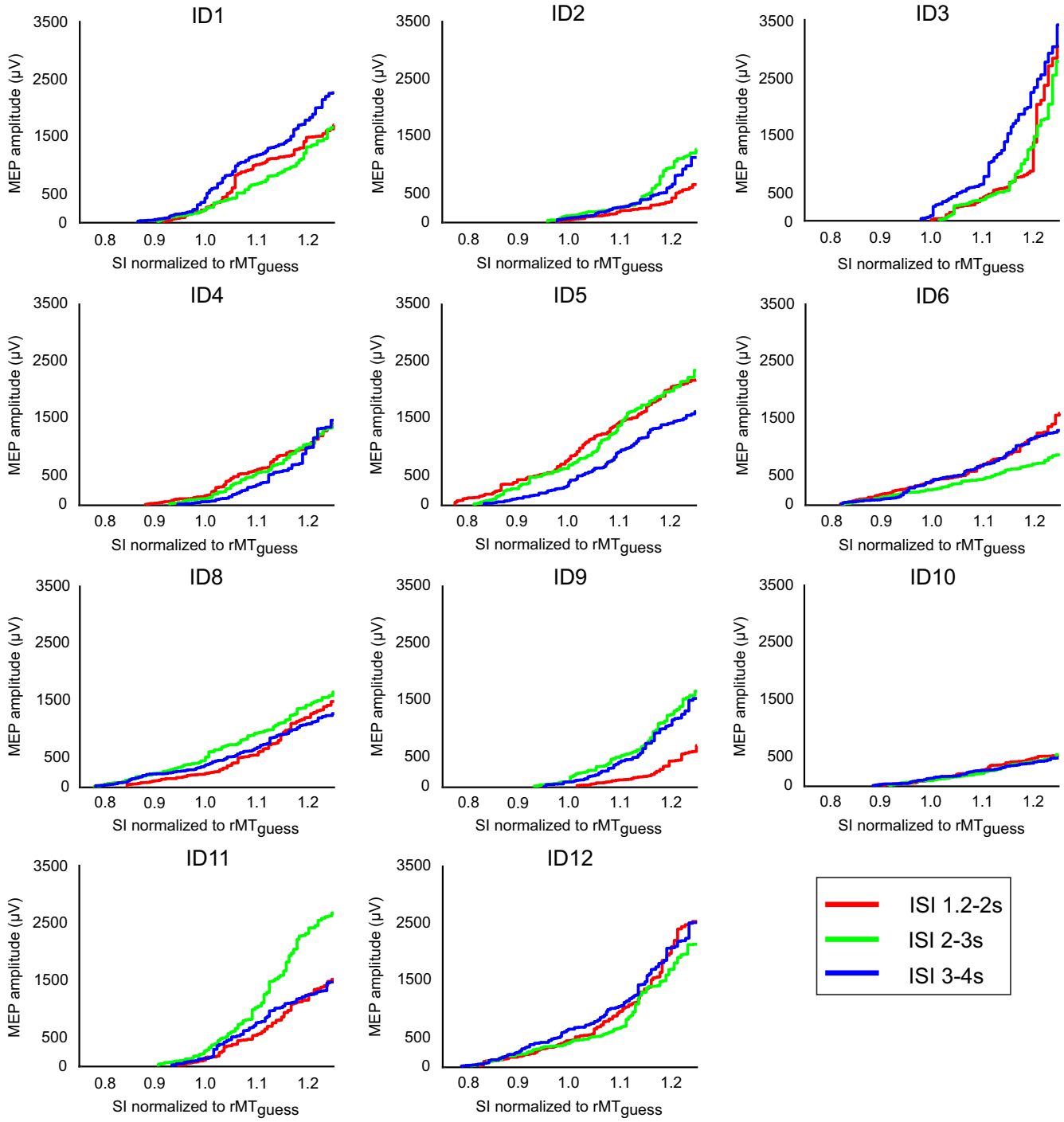


**Fig. 3.** Individual stimulus–response characteristics for each interstimulus interval (ISI). The order of the curves, from left-to-right, varies between subjects, and no clear trends exist. The further left the curve, the more excitable the curve is and vice versa.

varied from stimulus to stimulus, meaning that subthreshold SIs were included among the suprathreshold SI stimuli. Thus, it is plausible that short-interval intracortical inhibition or short-interval cortical facilitation occurred with some pulses and individuals. Previously, using random SI order has not, however, been observed to increase MEP variability compared to non-random order, although this result was obtained with an ISI longer than used in the present study (Möller et al., 2009). In fact, when using stimulus–response curves, the SIs need to be provided in random order due to hysteresis effects (Möller et al., 2009). Hysteresis reflects that the previous pulse alters the corticospinal excitability impacting the response of the next pulse and, thus, is associated with how neurons recover from the pulse. If the SIs are applied in descending strength, with an ISI of 5 s, the stimulus–response curve shifts to the left, i.e., the curve shows greater excitability (Möller et al., 2009). If the intensities are used in ascending order, the curve shifts to the right, i.e., the curve shows decreased excitability (Möller et al., 2009). Although the rMTguess utilized to select the SIs for each subject was estimated with only 3 pulses, rMTguess did not differ from those estimated with the established methods, Rossini-Rothwell, and threshold-hunting. This is important because, by definition, rMT is an SI that induces an MEP in

**Table 1**
Resting motor thresholds (rMT, % of maximum stimulator output) acquired with stimulus–response curves with varying interstimulus intervals (ISIs), Rossini-Rothwell (Rossini et al., 2015), and threshold-hunting method (Awiszus, 2003).

| Subject ID | ISI 1.2–2 s | ISI 2–3 s | ISI 3–4 s | $rMT_{guess}$(ISI > 5 s) | Rossini-Rothwell (ISI > 5 s) | Threshold hunting (ISI > 5 s) |
|---|---|---|---|---|---|---|
| 1 | 50 | 50 | 49 | 53 | 49 | 52 |
| 2 | 40 | 39 | 40 | 39 | 38 | 38 |
| 3 | 36 | 35 | 34 | 33 | 36 | 35 |
| 4 | 41 | 42 | 44 | 43 | 42 | 43 |
| 5 | 38 | 40 | 46 | 53 | 46 | 44 |
| 6 | 37 | 36 | 37 | 43 | 33 | 35 |
| 8 | 40 | 37 | 36 | 45 | 37 | 36 |
| 9 | 40 | 37 | 37 | 37 | 38 | 37 |
| 10 | 58 | 59 | 57 | 63 | 61 | 60 |
| 11 | 40 | 39 | 38 | 40 | 38 | 39 |
| 12 | 37 | 37 | 36 | 42 | 31 | 35 |

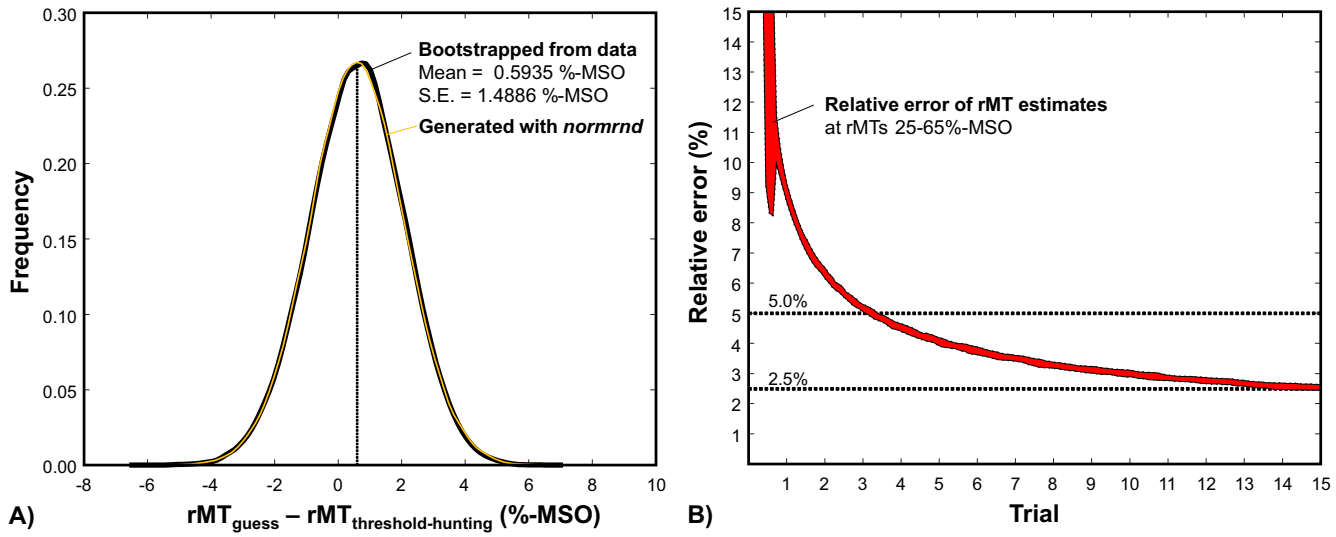


**Fig. 4.** A) Bootstrapped distribution of the difference between the $rMT_{guess}$ values and the rMTs (defined with the maximum stimulator output, MSO) determined using threshold-hunting based on the data provided in Table 1. These data were used to estimate the paradigm-induced error values based on Monte-Carlo simulations. The pre-defined stimulation intensities were computed based on generated $rMT_{guess}$ values with a randomized difference (with Matlab normrnd-function) to the $rMT_{true}$-value. B) Relative errors for estimated rMT-values based on stimulus–response curves as a function of the applied trial. Each trial included 11 stimuli at pre-defined stimulation intensities between 75% and 125% of $rMT_{guess}$, and simulations were conducted until 15 trials. These simulations do not account for between-subjects effects observed at short interstimulus intervals. *rMT = resting motor threshold, $rMT_{guess}$ = resting motor threshold estimated with three scout pulses and prior data.*

50% of the stimulations (Rossini et al., 2015). When using stimulus–response curves to estimate the rMT, the intensity selection should thus include both sub- and supra-threshold intensities. As rMTguess did not differ from the established methods at the group level, the intensity selection was considered not to be biased either towards sub- or supra-threshold SI at the group level, even if some biases might have occurred in individual subjects.

Other underlying factors causing the MEP amplitude distributions to depend on the ISI might be varying descending waves. Low TMS SIs typically stimulate neurons trans-synaptically producing I-waves in the descending motor pathways, whereas higher intensities generate a D-wave before the I-waves (Ziemann, 2020). The D- and I-waves sum up and activate contralateral motoneurons in the spinal cord that elicit an MEP in the target muscle. There are some indications that the I-wave synchrony increases with increasing SI (Pitcher et al., 2003). It may also be that the ISI influences the I-wave synchrony but differently across the subjects. Our previous findings support that I-wave effects, measured with MEPs, are highly variable between subjects (Kallioniemi et al., 2018). Repeated TMS pulses could also change neuronal states (Fedele et al., 2016) and brain dynamics (Stamoulis et al., 2011) differently across the subjects, translating to MEP variability between subjects.

Based on our simulations, the rMT could be estimated with 44 pulses without between-subjects variability in stimulus–response curves. However, in the experimental data, the short ISIs presumably changed threshold and/or threshold spread in an unpredictable way. Thus, the minimum number of stimuli for this paradigm obtained in the Monte-Carlo simulations is not valid for real data. It is expected that >100 pulses would be needed for a reliable rMT estimate, regardless of the ISI, with stimulus–response curves. In threshold-hunting, which is an already established rMT estimation approach, with an ISI of at least 5 s, only 18 pulses are required for a reliable rMT estimate (Awiszus, 2011). This reflects a total measurement time of 90 s with the threshold-hunting approach compared to >100 s with the stimulus–response curves. Hence, the time potentially gained with short ISIs in stimulus–response curves is lost in the number of pulses needed in comparison to already established methods. We cannot provide the minimum time, i.e., number of pulses, needed for rMT estimation with stimulus–response curves due to each participant reacting to the short ISIs differently and unexpectedly. This suggests that short ISIs should not be used to estimate rMTs to minimize experiment time. This is in line with previous studies performed at constant SI or with a very low number of stimuli (Awiszus, 2011; Qi et al., 2011; Julkunen et al., 2012).

Regardless of the inconsistency in how individuals reacted to short ISIs, the rMTs from stimulus-response curves and the more established methods, Rossini-Rothwell and threshold-hunting did not differ at the group level. As shown in Table 1, however, despite the similarities at the group level, there was variability in consistency at the individual level. In most of the participants, this variability was relatively low. Although the Rossini-Rothwell and threshold-hunting did not necessarily result in exactly the same value, it is more challenging to evaluate the underlying cause for this variability than that associated with stimulus–response curves. This is because Rossini-Rothwell and threshold-hunting only produce a single measure, the rMT, compared to stimulus–response curves that show a broad range of cortical excitability.

Short measurement times could be beneficial from several perspectives. During a TMS measurement, the participant needs to sit still, which may be challenging in some study populations. Also, in many study designs, the coil needs to be moved during the measurement, and thus, the coil needs to be held manually, which puts a strain on the researcher. High SIs may cause scalp discomfort, and long measurement times may be susceptible to changes in the physiological state of the participant. For example, subject alertness level (Mars et al., 2007; Noreika et al., 2020), arousal (Bell et al., 2018), pre-TMS muscle activity (Kiers et al., 1993; Darling et al., 2006), desynchronization of action potentials (Magistris et al., 1998), afferent feedback (Nielsen, 1996), acoustic noise (Löfberg et al., 2018) and ongoing electroencephalography oscillations (Schaworonkow et al., 2019) are known to modulate MEP amplitudes. Also, coil orientation and location stability (Brasil-Neto et al., 1992; Kallioniemi et al., 2015b; de Goede et al., 2018) influence the MEPs. From these perspectives, a shorter rMT estimation but also a shorter whole experiment time would be desirable. Earlier studies have reported that with an ISI of at least 5 s, 21 pulses are needed to stabilize MEP amplitude and 23 to stabilize MEP latency (Chang et al., 2016). These results were obtained with a constant SI and with a neuronavigation system, and similar results have been found without neuronavigation (Goldsworthy et al., 2016). It has been suggested that the minimum number of pulses is limited due to an initial transient-state in cortical excitability after stimulation is started (Schmidt et al., 2009). However, the exact physiological phenomenon behind this is still unknown (Schmidt et al., 2009).

Although our study provides substantial evidence against using short ISIs, our study did not evaluate ISIs longer than 4 s. Thus, we cannot recommend the shortest ISI needed to estimate the rMT via stimulus–response curves so that all subjects react to the stimulation similarly. This should be addressed in future studies, which should evaluate ISIs up to 15 s as MEP amplitudes were recently found to increase with increasing ISI up to 15 s (Hassanzahraee et al., 2019). Also, the MEP amplitude variability is known to decrease up to 15 s (Hassanzahraee et al., 2019). Furthermore, our study aimed to evaluate whether individuals react similarly to short ISIs and not to evaluate the frequency or underlying causes of different response patterns at a group- or population-level. To assess the response patterns more comprehensively, a future study with a higher number of participants is needed.

Although rMT is a relatively stable measure over time (Kimiskidis et al., 2004; Danner et al., 2008), it is somewhat impacted by the circadian rhythm (Huber et al., 2013). Thus, rMT estimated in the morning might slightly differ from that estimated in the evening. In our measurements, all stimulus–response curves in a subject were measured within a short time frame preventing the influence of different phases of the circadian rhythm at the individual level. Circadian rhythm, however, could have influenced the group level results as the measurement times were not standardized between subjects. The possibility for this is low, as the stimulus–response curves did not differ at the group level. For this same reason, the influence of any other factors potentially influencing cortical excitability, such as the number of hours slept during the previous night (Huber et al., 2013), is low. Finally, while the order of ISI within the stimulus–response curve experiments were randomized, the order of stimulus–response curve experiments and the more established rMT estimation methods were not randomized, i.e., the established methods were always applied in the end of the session. As there were no significant differences between any two rMTs, this did not significantly influence the results.

## 5. Conclusions

Acquiring the rMT quickly with a stimulus–response curve might help decrease the overall measurement duration, and subject discomfort. Reducing the ISI, however, impacts the rMT as we do not know yet what is the shortest ISI needed for the participants to react similarly. Accordingly, short ISIs should be used with caution. Despite the limitation with short ISIs, stimulus–response curves provide a useful and comprehensive view of cortical excitability and may complement pure rMT estimation methods, such as Rossini-Rothwell and threshold-hunting. Thus, stimulus–response curves should not be neglected in TMS research, but further research is needed to clarify the factors influencing them and their test–retest reliability.

## Conflict of Interest Statement

Petro Julkunen has a patent with Nexstim Plc (Helsinki, Finland), a manufacturer of navigated TMS systems. Minna Pitkänen is employed by Bittium Biosignals Ltd (Kuopio, Finland). The rest of the authors declare no competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cnp.2021.10.005.

## References

Awiszus, F., 2003. TMS and threshold hunting. Suppl. Clin. Neurophysiol. 56, 13–23. https://doi.org/10.1016/S1567-424X(09)70205-3.

Awiszus, F., 2011. Fast estimation of transcranial magnetic stimulation motor threshold: is it safe? Brain Stimul. 4 (1), 58–59. https://doi.org/10.1016/j.brs.2010.09.004.

Barker, A.T., Jalinous, R., Freeston, I.L., 1985. Non-invasive magnetic stimulation of human motor cortex. Lancet 325 (8437), 1106–1107. https://doi.org/10.1016/S0140-6736(85)92413-4.

Bell, S.J., Lauer, A., Lench, D.H., Hanlon, C.A., 2018. Visual attention affects the amplitude of the transcranial magnetic stimulation-associated motor-evoked potential: a preliminary study with clinical utility. J. Psychiatr. Pract. 24, 220–229. https://doi.org/10.1097/PRA.0000000000000321.

Brasil-Neto, J.P., Cohen, L.G., Panizza, M., Nilsson, J., Roth, B.J., Hallett, M., 1992. Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. J. Clin. Neurophysiol. 9 (1), 132–136.

Chang, W.H., Fried, P.J., Saxena, S., Jannati, A., Gomes-Osman, J., Kim, Y.-H., Pascual-Leone, A., 2016. Optimal number of pulses as outcome measures of neuronavigated transcranial magnetic stimulation. Clin. Neurophysiol. 127 (8), 2892–2897. https://doi.org/10.1016/j.clinph.2016.04.001.

Danner, N., Julkunen, P., Könönen, M., Säisänen, L., Nurkkala, J., Karhu, J., 2008. Navigated transcranial magnetic stimulation and computed electric field strength reduce stimulator-dependent differences in the motor threshold. J. Neurosci. Methods 174 (1), 116–122. https://doi.org/10.1016/j.jneumeth.2008.06.032.

Darling, W.G., Wolf, S.L., Butler, A.J., 2006. Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. Exp. Brain Res. 174 (2), 376–385. https://doi.org/10.1007/s00221-006-0468-9.

de Goede, A.A., ter Braack, E.M., van Putten, M.J.A.M., 2018. Accurate coil positioning is important for single and paired pulse TMS on the subject level. Brain Topogr. 31 (6), 917–930. https://doi.org/10.1007/s10548-018-0655-6.

Engelhardt, M., Schneider, H., Gast, T., Picht, T., 2019. Estimation of the resting motor threshold (RMT) in transcranial magnetic stimulation using relative-frequency and threshold-hunting methods in brain tumor patients. Acta Neurochir. (Wien) 161 (9), 1845–1851. https://doi.org/10.1007/s00701-019-03997-z.

Fedele, T., Blagovechtchenski, E., Nazarova, M., Iscan, Z., Moiseeva, V., Nikulin, V.V., 2016. Long-range temporal correlations in the amplitude of alpha oscillations predict and reflect strength of intracortical facilitation: combined TMS and EEG study. Neuroscience 331, 109–119. https://doi.org/10.1016/j.neuroscience.2016.06.015.

Goldsworthy, M.R., Hordacre, B., Ridding, M.C., 2016. Minimum number of trials required for within- and between-session reliability of TMS measures of corticospinal excitability. Neuroscience 320, 205–209. https://doi.org/10.1016/j.neuroscience.2016.02.012.

Hassanzahraee, M., Zoghi, M., Jaberzadeh, S., 2019. Longer transcranial magnetic stimulation intertrial interval increases size, reduces variability, and improves the reliability of motor evoked potentials. Brain Connect. 9 (10), 770–776. https://doi.org/10.1089/brain.2019.0714.

Huber, R., Mäki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A.G., Tononi, G., Massimini, M., 2013. Human cortical excitability increases with time awake. Cereb. Cortex 23, 332–338. https://doi.org/10.1093/cercor/bhs014.

Ilmoniemi, R.J., Ruohonen, J., Karhu, J., 1999. Transcranial magnetic stimulation–a new tool for functional imaging of the brain. Crit. Rev. Biomed. Eng. 27, 241–284.

Julkunen, P., 2019. Mobile application for adaptive threshold hunting in transcranial magnetic stimulation. IEEE Trans. Neural Syst. Rehabil. Eng. 27 (8), 1504–1510. https://doi.org/10.1109/TNSRE.2019.2925904.

Julkunen, P., Ruohonen, J., Sääskilahti, S., Säisänen, L., Karhu, J., 2011. Threshold curves for transcranial magnetic stimulation to improve reliability of motor pathway status assessment. Clin. Neurophysiol. 122 (5), 975–983. https://doi.org/10.1016/j.clinph.2010.09.005.

Julkunen, P., Säisänen, L., Hukkanen, T., Danner, N., Könönen, M., 2012. Does second-scale intertrial interval affect motor evoked potentials induced by single-pulse transcranial magnetic stimulation? Brain Stimul. 5 (4), 526–532. https://doi.org/10.1016/j.brs.2011.07.006.

Kallioniemi, E., Julkunen, P., 2016. Alternative stimulation intensities for mapping cortical motor area with navigated TMS. Brain Topogr. 29 (3), 395–404. https://doi.org/10.1007/s10548-016-0470-x.

Kallioniemi, E., Säisänen, L., Pitkänen, M., Könönen, M., Karhu, J., Julkunen, P., 2015a. Input-output characteristics of late corticospinal silent period induced by transcranial magnetic stimulation. J. Clin. Neurophysiol. 32 (4), 346–351. https://doi.org/10.1097/WNP.0000000000000177.

Kallioniemi, E., Könönen, M., Julkunen, P., 2015b. Repeatability of functional anisotropy in navigated transcranial magnetic stimulation–coil-orientation versus response. NeuroReport 26, 515–521. https://doi.org/10.1097/WNR.0000000000000380.

Kallioniemi, E., Savolainen, P., Järnefelt, G., Koskenkorva, P., Karhu, J., Julkunen, P., 2018. Transcranial magnetic stimulation modulation of corticospinal excitability by targeting cortical I-waves with biphasic paired-pulses. Brain Stimul. 11 (2), 322–326. https://doi.org/10.1016/j.brs.2017.10.014.

Kiers, L., Cros, D., Chiappa, K.H., Fang, J., 1993. Variability of motor potentials evoked by transcranial magnetic stimulation. Electroencephalogr. Clin. Neurophysiol. 89 (6), 415–423. https://doi.org/10.1016/0168-5597(93)90115-6.

Kimiskidis, V.K., Papagiannopoulos, S., Sotirakoglou, K., Kazis, D.A., Dimopoulos, G., Kazis, A., Mills, K.R., 2004. The repeatability of corticomotor threshold measurements. Clin. Neurophysiol. 34 (6), 259–266. https://doi.org/10.1016/j.neucli.2004.10.002.

Konakanchi, D., de Jongh Curry, A.L., Waters, R.S., Narayana, S., 2020. Focality of the induced E-field is a contributing factor in the choice of TMS parameters: evidence from a 3D computational model of the human brain. Brain Sci. 10, 1010. https://doi.org/10.3390/brainsci10121010.

Kukke, S.N., Paine, R.W., Chao, C.-C., de Campos, A.C., Hallett, M., 2014. Efficient and reliable characterization of the corticospinal system using transcranial magnetic stimulation. J. Clin. Neurophysiol. 31, 246–252. https://doi.org/10.1097/WNP.0000000000000057.

Lefaucheur, J.-P., Aleman, A., Baeken, C., Benninger, D.H., Brunelin, J., Di Lazzaro, V., Filipović, S.R., Grefkes, C., Hasan, A., Hummel, F.C., Jääskeläinen, S.K., Langguth, B., Leocani, L., Londero, A., Nardone, R., Nguyen, J.-P., Nyffeler, T., Oliveira-Maia, A.J., Oliviero, A., Padberg, F., Palm, U., Paulus, W., Poulet, E., Quartarone, A., Rachid, F., Rektorová, I., Rossi, S., Sahlsten, H., Schecklmann, M., Szekely, D., Ziemann, U., 2020. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). Clin. Neurophysiol. 131 (2), 474–528. https://doi.org/10.1016/j.clinph.2019.11.002.

Löfberg, O., Julkunen, P., Kallioniemi, E., Pääkkönen, A., Karhu, J., 2018. Modulation of motor cortical excitability with auditory stimulation. J. Neurophysiol. 120 (3), 920–925. https://doi.org/10.1152/jn.00186.2017.

Magistris, M.R., Rösler, K.M., Truffert, A., Myers, J.P., 1998. Transcranial stimulation excites virtually all motor neurons supplying the target muscle. A demonstration and a method improving the study of motor evoked potentials. Brain 121, 437–450. https://doi.org/10.1093/brain/121.3.437.

Mars, R.B., Bestmann, S., Rothwell, J.C., Haggard, P., 2007. Effects of motor preparation and spatial attention on corticospinal excitability in a delayed-response paradigm. Exp. Brain Res. 182 (1), 125–129. https://doi.org/10.1007/s00221-007-1055-4.

Mathias, J.P., Barsi, G.I., van de Ruit, M., Grey, M.J., 2014. Rapid acquisition of the transcranial magnetic stimulation stimulus response curve. Brain Stimul. 7 (1), 59–65. https://doi.org/10.1016/j.brs.2013.08.003.

Mills, K.R., Nithi, K.A., 1997. Corticomotor threshold to magnetic stimulation: normal values and repeatability. Muscle Nerve 20, 570–576. https://doi.org/10.1002/(SICI)1097-4598(199705)20:5<570::AID-MUS5>3.0.CO;2-6.

Möller, C., Arai, N., Lücke, J., Ziemann, U., 2009. Hysteresis effects on the input–output curve of motor evoked potentials. Clin. Neurophysiol. 120 (5), 1003–1008. https://doi.org/10.1016/j.clinph.2009.03.001.

Nielsen, J.F., 1996. Logarithmic distribution of amplitudes of compound muscle action potentials evoked by transcranial magnetic stimulation. J. Clin. Neurophysiol. 13 (5), 423–434. https://doi.org/10.1097/00004691-199609000-00005.

Noreika, V., Kamke, M.R., Canales-Johnson, A., Chennu, S., Bekinschtein, T.A., Mattingley, J.B., 2020. Alertness fluctuations when performing a task modulate cortical evoked responses to transcranial magnetic stimulation. NeuroImage 223, 117305. https://doi.org/10.1016/j.neuroimage.2020.117305.

Pellicciari, M.C., Miniussi, C., Ferrari, C., Koch, G., Bortoletto, M., 2016. Ongoing cumulative effects of single TMS pulses on corticospinal excitability: An intra- and inter-block investigation. Clin. Neurophysiol. 127 (1), 621–628. https://doi.org/10.1016/j.clinph.2015.03.002.

Pitcher, J.B., Ogston, K.M., Miles, T.S., 2003. Age and sex differences in human motor cortex input-output characteristics. J. Physiol. 546 (2), 605–613. https://doi.org/10.1113/jphysiol.2002.029454.

Pitkänen, M., Kallioniemi, E., Julkunen, P., Chen, R., 2017. Effect of inter-train interval on the induction of repetition suppression of motor-evoked potentials using transcranial magnetic stimulation. PLoS ONE 12 (7), e0181663. https://doi.org/10.1371/journal.pone.0181663.

Pretalli, J.-B., Nicolier, M., Chopard, G., Vandel, P., Tio, G., Monnin, J., Pazart, L., Sechter, D., Haffen, E., 2012. Resting motor threshold changes and clinical response to prefrontal repetitive transcranial magnetic stimulation in depressed patients. Psychiatry Clin. Neurosci. 66 (4), 344–352. https://doi.org/10.1111/j.1440-1819.2012.02341.x.

Qi, F., Wu, A.D., Schweighofer, N., 2011. Fast estimation of transcranial magnetic stimulation motor threshold. Brain Stimul. 4 (1), 50–57. https://doi.org/10.1016/j.brs.2010.06.002.

Romero, J.R., Anschel, D., Sparing, R., Gangitano, M., Pascual-Leone, A., 2002. Subthreshold low frequency repetitive transcranial magnetic stimulation selectively decreases facilitation in the motor cortex. Clin. Neurophysiol. 113 (1), 101–107. https://doi.org/10.1016/S1388-2457(01)00693-9.

Rossi, S., Hallett, M., Rossini, P.M., Pascual-Leone, A., 2009. Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin. Neurophysiol. 120 (12), 2008–2039. https://doi.org/10.1016/j.clinph.2009.08.016.

Rossini, P.M., Burke, D., Chen, R., Cohen, L.G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P.B., George, M.S., Hallett, M., Lefaucheur, J.P., Langguth, B., Matsumoto, H., Miniussi, C., Nitsche, M.A., Pascual-Leone, A., Paulus, W., Rossi, S., Rothwell, J.C., Siebner, H.R., Ugawa, Y., Walsh, V., Ziemann, U., 2015. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N Committee. Clin. Neurophysiol. 126 (6), 1071–1107. https://doi.org/10.1016/j.clinph.2015.02.001.

Säisänen, L., Julkunen, P., Niskanen, E., Danner, N., Hukkanen, T., Lohioja, T., Nurkkala, J., Mervaala, E., Karhu, J., Könönen, M., 2008. Motor potentials evoked by navigated transcranial magnetic stimulation in healthy subjects. J. Clin. Neurophysiol. 25 (6), 367–372. https://doi.org/10.1097/WNP.0b013e31818e7944.

Sankarasubramanian, V., Roelle, S.M., Bonnett, C.E., Janini, D., Varnerin, N.M., Cunningham, D.A., Sharma, J.S., Potter-Baker, K.A., Wang, X., Yue, G.H., Plow, E. B., 2015. Reproducibility of transcranial magnetic stimulation metrics in the study of proximal upper limb muscles. J. Electromyogr. Kinesiol. 25 (5), 754–764. https://doi.org/10.1016/j.jelekin.2015.05.006.

Schaworonkow, N., Triesch, J., Ziemann, U., Zrenner, C., 2019. EEG-triggered TMS reveals stronger brain state-dependent modulation of motor evoked potentials at weaker stimulation intensities. Brain Stimul. 12 (1), 110–118. https://doi.org/10.1016/j.brs.2018.09.009.

Schmidt, S., Cichy, R.M., Kraft, A., Brocke, J., Irlbacher, K., Brandt, S.A., 2009. An initial transient-state and reliable measures of corticospinal excitability in TMS studies. Clin. Neurophysiol. 120 (5), 987–993. https://doi.org/10.1016/j.clinph.2009.02.164.

Sollmann, N., Tanigawa, N., Bulubas, L., Sabih, J., Zimmer, C., Ringel, F., Meyer, B., Krieg, S.M., 2017. Clinical factors underlying the inter-individual variability of the resting motor threshold in navigated transcranial magnetic stimulation

motor mapping. Brain Topogr. 30 (1), 98–121. https://doi.org/10.1007/s10548-016-0536-9.
Stamoulis, C., Oberman, L.M., Praeg, E., Bashir, S., Pascual-Leone, A., 2011. Single pulse TMS-induced modulations of resting brain neurodynamics encoded in EEG phase. Brain Topogr. 24 (2), 105–113. https://doi.org/10.1007/s10548-010-0169-3.
Strigaro, G., Hamada, M., Cantello, R., Rothwell, J.C., 2016. Variability in response to 1Hz repetitive TMS. Clin. Neurophysiol. 127 (3), e40. https://doi.org/10.1016/j.clinph.2015.11.126.
ter Braack, E.M., de Goede, A.A., van Putten, M.J.A.M., 2019. Resting motor threshold, MEP and TEP variability during daytime. Brain Topogr. 32 (1), 17–27. https://doi.org/10.1007/s10548-018-0662-7.
Touge, T., Gerschlager, W., Brown, P., Rothwell, J.C., 2001. Are the after-effects of low-frequency rTMS on motor cortex excitability due to changes in the efficacy of cortical synapses? Clin. Neurophysiol. 112 (11), 2138–2145. https://doi.org/10.1016/S1388-2457(01)00651-4.
Tranulis, C., Guéguen, B., Pham-Scottez, A., Vacheron, M.N., Cabelguen, G., Costantini, A., et al., 2006. Motor threshold in transcranial magnetic stimulation: comparison of three estimation methods. Clin. Neurophysiol. 36, 1–7. https://doi.org/10.1016/j.neucli.2006.01.005.
Weber, M., Eisen, A.A., 2002. Magnetic stimulation of the central and peripheral nervous systems. Muscle Nerve 25 (2), 160–175. https://doi.org/10.1002/mus.10038.
Ziemann, U., 2020. I-waves in motor cortex revisited. Exp. Brain Res. 238 (7-8), 1601–1610. https://doi.org/10.1007/s00221-020-05764-4.