Comparing electromyography, accelerometry, and visual inspection to assess the resting motor threshold for transcranial magnetic stimulation

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Abstract

Introduction: Electromyography (EMG) remains the gold standard for estimating the Resting Motor Threshold (RMT) in Transcranial Magnetic Stimulation (TMS) studies, but its cost and limited accessibility often lead researchers to use visual inspection (VIS). However, VIS may introduce variability and systematic bias. Accelerometry (ACC) offers a cost-effective, objective alternative to capture TMS-evoked responses.

Objective: To compare the RMT as estimated using EMG, ACC, and VIS.

Methods: Five participants underwent TMS while EMG, ACC, and video recordings were collected. Separately, 64 observers judged hand movement in videos to estimate RMT via VIS. RMTs were compared across the three methods using Bayesian model comparison, Bland-Altman analyses, and Intraclass Correlation Coefficients (ICCs).

Results: RMTs estimated via EMG were lower than those obtained using either ACC or VIS. Compared to EMG, VIS tended to overestimate RMT (mean bias = 5.23%, 95%CI = [1.00–11.00]), while ACC and VIS estimates were more closely aligned (mean bias = 0.43%, 95%CI = [–4.00 – 5.00]). ICC (2,1) values indicated moderate reliability for VIS vs EMG (mean = 0.580, 95%CI = [0.389 – 0.748]), and good-to-excellent reliability for VIS vs ACC (mean = 0.845). However, bootstrapped 95% confidence intervals identified significant variability in the estimates provided by visual inspection, ranging from +1 to +11 for VIS vs EMG, but as low as -4 to +5 for VIS vs ACC.

Conclusions: EMG remains the most sensitive technique for estimating the RMT, but when EMG is not feasible, accelerometery provides a quantifiable, more objective, and less variable alternative than visual inspection.

Keywords

Transcranial Magnetic Stimulation, Electromyography, Accelerometry, Visual Inspection

Highlights

- Visual inspection introduces observer-related variability in RMT estimation
- Visual inspection & accelerometry systematically overestimate RMT compared to EMG
- EMG remains the most sensitive method for estimating RMT
- Accelerometry is an objective alternative to visual inspection if EMG is unavailable

1. Introduction

Research on the effects of Transcranial Magnetic Stimulation (TMS) has largely focused on the primary motor cortex of the human brain (1). When an area of the primary motor cortex linked to a target muscle is stimulated, it can activate the corticospinal pathway leading to muscle contractions, and potentially induce movements in that muscle. By employing electromyography (EMG), the resulting muscle activity from this stimulation can be recorded as a 'Motor Evoked Potential' or MEP (2). Among the features of the MEP, the most commonly analysed is its amplitude, often measured from peak to peak in microvolts (μV). Measuring MEP amplitudes is central to defining the intensity of TMS that is delivered in most TMS studies, which is typically defined based on the Resting Motor Threshold (RMT) — the minimal stimulator output necessary to produce MEPs with an amplitude of ≥50μV in at least 5 out of 10 trials.

Although much of the early research on TMS has focused on fundamental motor responses, over time, TMS has garnered significant attention for its wide-ranging applications across diverse fields. In neurology, TMS has been used to examine conditions such as multiple sclerosis (3–7), stroke (8,9), epilepsy (10,11), Parkinson's disease (12–15), dystonia (16–18) and myopathy (19–21). The application of repetitive TMS has demonstrated substantial promise in treating psychiatric disorders, leveraging its ability to modulate neural activity in targeted brain regions. Conditions such as major depressive disorder (MDD) (22–24), obsessive-compulsive disorder (OCD) (23,25–27), anxiety(28), post-traumatic stress disorder (PTSD)(29,30) and schizophrenia (31,32) have all benefited from TMS interventions.

Additionally, its efficacy extends to behavioral disorders, including addiction (33), attention-deficit/hyperactivity disorder (ADHD) (34), and eating disorders (35). Beyond these possible therapeutic applications, TMS has also proven to be a valuable tool in cognitive research, to improve our understanding of cognitive processes such as mental imagery, decision making and action preparation (36). Thus, while TMS was initially developed to probe motor system function through the measurement of MEPs, it has since evolved into a versatile tool with broad applications.

Regardless of the exact field of study, EMG remains the gold standard for assessing TMS responses. However, EMG presents several drawbacks. The initial cost of EMG equipment such as apparatus and amplifiers, combined with recurring expenses for consumables like electrodes, electrode gel, and cleaning alcohol, makes it a relatively expensive technique (37). Additionally, precise electrode placement requires understanding of muscle anatomy and physiology (38). Preparatory steps, including skin cleaning, hair removal, and verifying the clarity of the signal, all add to the time required to create optimal recordings using this method (39). Despite its widespread use among research teams investigating motor control and neurophysiology, many laboratories employ EMG solely to evaluate the RMT (40–42). However, due to the costs and preparation time associated with EMG, some groups instead measure the "visually determined" RMT. This approach defines RMT as the lowest stimulator output that generates a visible movement (usually of the hand) in at least 5 out of 10 trials (43). While more accessible and less expensive than using EMG, the visual inspection method is inherently subjective (43) and therefore subject to inter- and intra-individual variability; moreover, it focuses on identifying the presence/absence

93 of movement without providing other quantifiable measures (e.g. the speed or
94 magnitude of the induced movement). Furthermore, previous reports provide
95 conflicting accounts of the relationship between measures collected using visual
96 inspection and EMG, with research indicating the visually defined threshold can differ
97 from that identified using EMG by between −10 to +2% of maximal stimulator output
98 (43,44). This is notable as under- or over-estimating the RMT could in turn lead to the
99 application of stimulation intensities that are too low to be effective, or that may
100 exceed TMS safety guidelines (45). Together, these limitations underscore the need
101 to investigate alternative methods to EMG that are both cost-effective and efficient,
102 but also allow precise quantification of TMS evoked responses.

103 Accelerometry emerges as a promising solution to address the issues with EMG and
104 visual inspection in assessing TMS responses. Specifically, accelerometry is more
105 cost-effective than EMG, requiring smaller initial outlays and incurring lower
106 recurring expenses. Accelerometry also enables researchers to objectively quantify
107 TMS responses, and is therefore likely to be more reliable than the subjective
108 assessments provided by visual inspection. Past studies have used accelerometry to
109 examine how TMS-evoked movement direction and magnitude change with training
110 (46–48). Recently, our laboratory has explored using accelerometry as a tool to
111 measure different characteristics of TMS evoked responses; although accelerometry
112 tends to overestimate the RMT, comparisons of measurements of MEP amplitudes
113 and accelerometer jerk readings were highly correlated, demonstrating this
114 measurement tool shows promising potential (49).

In this paper, the aim is to investigate and compare the measurement of the Resting Motor Threshold (RMT) using three different methods: electromyography (EMG), accelerometry (ACC), and visual inspection (VIS). By evaluating the accuracy, reliability, and practical application of each technique, the present study will determine their respective strengths and limitations in capturing the RMT.

2. Materials and Methods

All code and data are available on the following OSF repository : https://osf.io/2mpxf/.

2.1. General Design

The study leveraged a mixed-model design to examine data from a total sample of 105 participants. The final sample after application of exclusion criteria (see below) included 69 participants. This final sample comprised two groups of participants.

Data from Group 1 provided direct recordings of responses to TMS. Data collection involved recording data from five participants using three methods: electromyography, accelerometry, and video recordings of the hand during TMS (i.e. recordings demonstrating whether the intensity of TMS was sufficient to produce movement or not).

Data from Group 2 then focused on the visual determination of the resting motor threshold by individual observers. Participants in this second group observed video recordings taken from the first group, providing responses to indicate whether they observed a movement in each trial in response to TMS.

2.2. Group 1: Recording of responses to TMS (EMG, Accelerometry, and Video)

2.2.1. Participants

Five participants (2 females, 3 males), all right-handed, with an average age of 24.2 years (ranging from 21 to 29 years, SD = ± 2.31) took part in this part of the study.

141 The safety screening checklist recommended by Lefaucheur et al. (50) was
142 administered prior to stimulation to confirm participant eligibility and ensure safe
143 application for the use of TMS. The study was approved by the Ethics Committee
144 Saint-Luc Hospital, UCLouvain (2020/30JUL/389 - DNMT-TMS – amendment 2), and
145 each participant provided written informed consent prior to the experiment.
146 Participants also consented to video recordings of their hands to be presented to
147 other participants (see below).

148 2.2.2. Transcranial Magnetic Stimulation (TMS)

149 TMS was administered using a monophasic Magstim 200[2] stimulator with a figure-of-
150 eight coil (2 x Ø70 mm) directed posteriorly and toward the ipsilateral side with a
151 random interval of 4 to 6 seconds between each stimulation. To ensure precise
152 targeting, participants wore a head tracker (NT-103) and the Visor2 neuronavigation
153 system (ANT Neuro, Netherlands) was used with the standard brain model, which
154 provided real-time guidance for accurate coil positioning.

155 2.2.3. Electromyography (EMG)

156 EMG was utilized to record Motor Evoked Potentials (MEPs) from the First Dorsal
157 Interosseus (FDI) muscle of the index finger on the dominant hand. Before placing the
158 electrodes, the skin was cleaned with an alcohol solution and shaved if necessary to
159 remove hair and enhance conductivity. Two self-adhesive, pre-gelled bipolar surface
160 electrodes (Blue Sensor N, Ambu®, Denmark) were positioned on the muscle body
161 and its distal insertion. A reference electrode was placed on the styloid process of the

ulna. EMG signals were sampled at 2 kHz with an online digital notch filter (50Hz), bandpass filtered at 20–450Hz, and amplified using a D360 8-Channel Patient Amplifier (Digitimer®, England).

2.2.4. Accelerometer Recordings

A 8791A250 K-Shear® Miniature Triaxial Accelerometer (Kistler, USA) was secured to the nail of the index finger using micropore adhesive tape (Fig. 1). The accelerometer captured movement in the x, y, and z axes, enabling a complete reconstruction of the motion. The data were collected using a 4-Channel PiezoSmart® (TEDS) Power Supply/Signal Conditioner (Kistler, USA) and recorded as the finger's acceleration (m/s^2). Prior to analysis, the data were low-pass filtered offline using a 20 Hz 4th order Butterworth filter.

2.2.5. Video recordings

Video recordings of the experiment were captured using a GoPro Hero 8 Black, set to record at 1080p resolution and 120 frames per second. The camera was mounted on a tripod, positioned to film the hand from above and from the contralateral side (see Fig. 1). This setup was chosen to simulate the perspective of an experimenter during a typical experimental session and offered a clear view of the hand.

2.2.6. Protocol Design

Participants sat on a chair with the palmar side of the dominant hand resting face down on the table, and were able to make adjustments until they found a comfortable

182 position that they could maintain throughout testing. The corticomotor area over the
183 left hemisphere corresponding to the right first dorsal interosseous (FDI) muscle was
184 identified, and the motor hotspot was determined using a figure-of-eight coil (2 × Ø70
185 mm) held tangentially to the scalp and positioned at approximately 45° to the sagittal
186 midline, with the handle pointing posteriorly and laterally to induce a posterior–
187 anterior current in the cortex, which is the standard orientation for FDI hotspotting
188 (51,52). The coil placement is determined by record stimulation sites based on the
189 standard MRI template embedded in the software. A theoretical FDI hotspot was first
190 located within the hand area of the motor cortex. The coil was then systematically
191 moved in 0.5 cm steps in posterior–anterior and medial–lateral directions until the
192 optimal scalp position eliciting the largest and most MEPs were identified. The final
193 hotspot coordinates were stored within the neuronavigation system to ensure
194 consistent coil placement throughout the session. RMT was assessed using EMG,
195 defined as the lowest intensity of maximal stimulator output (MSO) to produce a MEP
196 of ≥50μV in at least 5 out of 10 trials. Stimulation was randomly applied at intensities
197 ranging from -5%MSO to +20%MSO of the EMG-defined RMT. An external
198 experimenter (A.L.) visually monitored the participant's hand and signaled to the
199 experimenter delivering TMS (G.H.) if an intensity of TMS that produced a movement
200 on each trial (i.e. 10/10 trials) was observed, and future intensities of stimulation
201 were applied only below this intensity. This procedure identified the lowest
202 stimulator intensity above the EMG-defined RMT that consistently elicited visible
203 movements in all trials (i.e., 10/10)(range: +9% to +19% MSO).

2.2.7. Data Processing

EMG and accelerometer data were recorded using a Power1401–3A system and Signal software (both from Cambridge Electronic Design, England). The data were processed using custom MATLAB scripts.

EMG signal analysis focused on identifying the 'peak-to-peak' amplitude of the Motor Evoked Potential (MEP), which was calculated as the difference between the highest positive and negative peaks of the response in a post-stimulation window of 200ms.

Accelerometer data, capturing movements in the x, y, and z axes, were combined using the Cartesian formula (absolute acceleration = $\sqrt{(x^2 + y^2 + z^2)}$) to generate a single value representing the total acceleration of the sensor (i.e. a measure sensitive to any movement of the finger in response to TMS, analogous to visual inspection). The derivative of the acceleration was then calculated to determine the 'jerk' (m/s^3), with the absolute peak jerk being the primary signal of interest. Jerk is particularly useful for analysing rapid or transient movements, which are common in TMS-induced responses.

The processed accelerometer data were also used to identify the RMT as determined by accelerometry. The RMT was defined as the intensity of the maximal stimulator output (MSO) at which at least 5 out of 10 trials exhibited a peak jerk (m/s^3) exceeding the 95% confidence interval calculated from the 200 ms preceding the TMS stimulation (49)(i.e., a baseline, motionless period for each participant).

225 **2.3. Group 2: Video observations to determine the visual RMT**

226 2.3.1. Participants

227 Initially, 100 participants were recruited through a combination of in-person sessions
228 (20 participants) and the Prolific online platform (80 participants). However, after
229 data verification and attention checks were considered (see below), a total of 64
230 participants were retained for analysis. These 64 participants (33 females, 31 males)
231 had an average age of 34.1 years (range: 18–62 years, SD = ±9.4 - for full descriptive
232 data for the final sample see Supplementary Table 1). The study was approved by the
233 Ethics Committee Saint-Luc Hospital, UCLouvain (2020/30JUL/389 - DNMT-TMS –
234 amendment 3), and participants gave informed consent though a computerized form.
235 Participants received compensation of up to 8€ for their time.

236 2.3.2. Procedure and stimuli

237 The experimental protocol was implemented using PsychoPy version 2024.4, running
238 on Python 3.10 (53) and transformed to PsychoJS experiment to be hosted online on
239 Pavlovia servers (54).

240 The experiment began with initial screens that provided instructions to the observer
241 (see supplementary materials - Video instruction). Each trial of the experiment then
242 began with a preparation screen (see Figure 1), displaying a fixation cross in the
243 center of the screen for 1 second. The main stimuli in this experiment consisted of
244 short video clips, each lasting 2 seconds, with a single pulse of TMS being delivered at
245 a specific intensity 1 second into the video. Following the video, participants were

246 given a 10-second response window in which they answered "yes" (right arrow) "no"
247 (left arrow) to indicate whether they observed any movement in the hand. In the case
248 of a technical issue with the stimulus presentation (video freezing, images or sound
249 missing), participants could select the option “problem” (down arrow), which would
250 mark the trial for removal from any subsequent analysis.

251 After the initial instructions were provided, participants observed two example
252 videos: one without any movement in response to TMS, and one with a pronounced
253 movement in response to TMS. Participants then completed blocks in which they
254 observed 10 trials where TMS was delivered at a fixed intensity of MSO. The intensity
255 assigned to each block was determined randomly by the program, so that the order
256 of presentation did not follow a progressive or predictable sequence. At the end of
257 each block (i.e., after viewing all 10 videos for a given intensity level), participants
258 encountered a pause screen and were able to rest as long as they required before
259 beginning the next block of trials.

260 Each participant in Group 2 was randomly assigned to view videos taken from one of
261 the participants in Group 1 (from this point onwards we refer to participants in Group
262 2 as “observers” to help distinguish them from participants in Group 1). Notably, the
263 number of trials required to produce a clear visual response in all trials at a fixed
264 intensity varied from participant to participant in Group 1. As a result, the total
265 number of videos available for observation also differed across participants in Group
266 2, meaning that not all observers viewed the exact same video set. Importantly,
267 however, all observers completed the full set of videos assigned to them. Observers

saw trials delivered across at least 15 intensities, and a maximum 20 intensities (average 17.8 intensities – see also table 1 for summary information). Every 5 blocks, an attention task appeared and asked the observer to press the "up" arrow during both offline or online session; this attention check was designed to be able to catch any participants who were not following the instructions given (e.g. simply repeatedly pressing the same response button across all trials, or randomly selecting one of the three buttons that were normally used to provide a response).

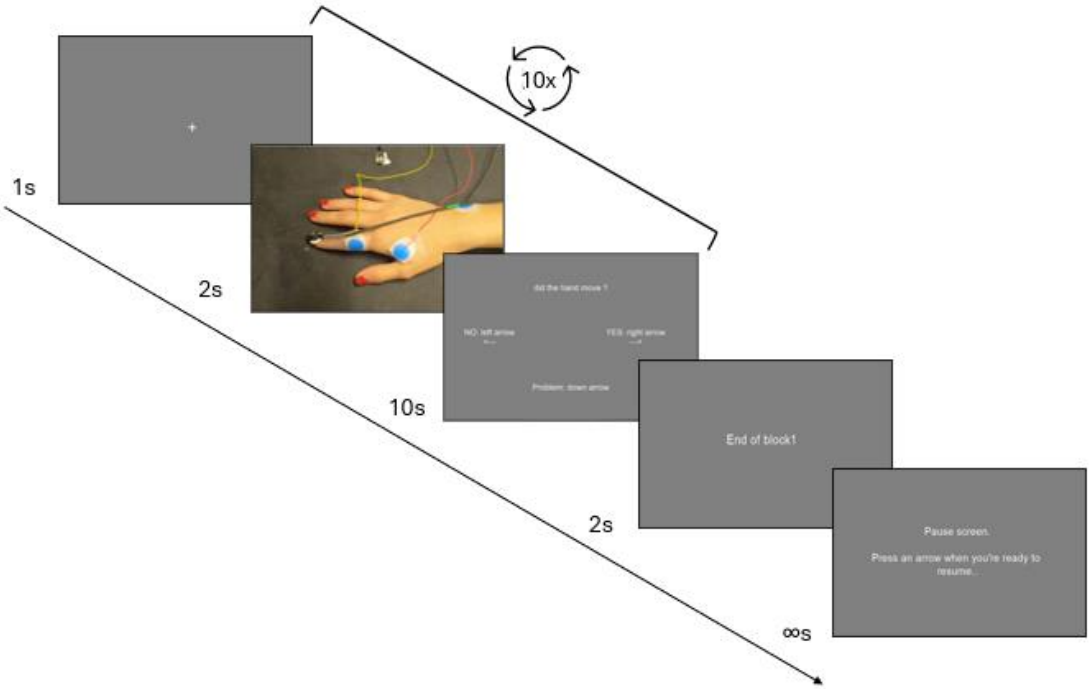


Figure 1 : Trial structure for the visual inspection task.

278 2.3.3. Data processing

279 For each observer, the number of "movement" and "non-movement" responses were
280 recorded. These responses were then collapsed across each intensity level to
281 determine the RMT, defined as the intensity at which the proportion of trials with a
282 "movement" response reached or exceeded 50%. This approach is equivalent to the
283 standard EMG/visual method but accounts for any trials that were removed due to
284 technical errors. In total 34 trials were removed (i.e. 0.3% of all trials presented).

285 **2.4. Statistical analyses**

286 2.4.1. Bayesian ANOVA

287 A Bayesian ANOVA was implemented to analyze the effect of the conditions on the
288 dependent variable RMT. This approach allows for the estimation for the effects of
289 conditions (EMG, ACC, and VIS) while accounting for model uncertainty. Random
290 effects, including video series (i.e. different participants from Group 1) and observers
291 (i.e. different participants from Group 2), were incorporated to control inter-
292 individual variability. To interpret the Bayes factors (BFs), statistical analysis
293 followed the classification proposed by Lee & Wagenmakers (55). A grouped scatter
294 plot was created to visualize the data distribution and inter-individual variability in
295 RMT measurements. To further refine the analysis, a Model-Averaged Posterior
296 Summary was conducted, providing weighted mean estimates of condition effects
297 while integrating model selection uncertainty. The posterior mean, standard

deviation, and 95% credible intervals were extracted for each condition (EMG, ACC, VIS) to offer a more robust evaluation of their impact on RMT.

Bland-Altman Plots

Separate Bland-Altman Plots were generated to examine the agreement between EMG (i.e. the gold standard) and ACC, as well as EMG and VIS, by analyzing systematic biases and limits of agreement. The Bland-Altman plot takes the difference between two measures plotted against their mean, and is therefore useful to observe systematic bias from one method to the other. This visualization was essential for identifying potential measurement discrepancies between the different methods, and assessing their level of agreement. For data examining VIS conditions, we calculated 95% confidence intervals based on a bootstrapping procedure. For each of the video series presented (i.e. data from a single participant in Group 1), 10,000 resamples of the corresponding VIS data (i.e. observers from Group 2 who saw videos from that specific participant in Group 1) were generated with replacement. The mean for each resample was then taken, allowing us to identify a 95% confidence interval (i.e. the 2.5th and 97.5th percentiles). This approach thus ensured that the analyses were not biased by unequal observer contributions or systematic rating sequences, allowing us to obtain more robust estimations of the effects across conditions.

2.4.2. Intraclass Correlation Coefficient (ICC) analyses

Several reliability and agreement analyses were performed to model condition effects. First, Intraclass Correlation Coefficient (ICC) with a two-way random effect

319 model of absolute agreement and single measures (i.e. ICC(2,1), used to evaluate the
320 agreement across raters/measurements) was computed to assess the absolute
321 agreement between EMG (gold standard) and VIS, considering differences across
322 observers(56). Given that the number of observers varied between participants and
323 video series, we implemented a bootstrap-based resampling procedure. We
324 resampled the data 10,000 times, such that each of the 5 different video series
325 (depicting data from Group 1) was assessed by one randomly selected observer (i.e.
326 a single participant from Group 2 was selected from all those available who had
327 watched the corresponding video series). The ICC(2,1) was then calculated for each
328 resample. This approach allowed us to calculate both the mean value, and a
329 bootstrapped 95% confidence interval (taking the 2.5% and the 97.5% percentile ICC
330 values), allowing us to generate a more detailed estimation of the distribution of ICC
331 values. To verify that this resampling did not bias the results, an ICC analysis based
332 on the mean ratings per condition (i.e. ICC (2,k)) analysis with a two-way random
333 effect model of absolute agreement and multiple measures was also calculated and
334 compared with the ICC (2,1) confidence interval.

335 Additionally, an ICC (2,1) was computed to assess agreement between ACC and VIS
336 conditions, following the same bootstrapping resampling to ensure robustness
337 despite the variability in observer numbers. The ICC (2,k) was computed following
338 the same methodology as described above.

339 Finally, the ICC (2,1) was computed to assess agreement between ACC and EMG
340 conditions following without bootstrapping due to the number of data acquired (no

ICC (2,k) analysis was performed as only one measure was available per participant in Group 1).

To establish reliability thresholds, analysis referred to the classification proposed in Koo & Li (2016) (56) as values below 0.5 indicate poor reliability, those between 0.5 and 0.75 reflect moderate reliability, values ranging from 0.75 to 0.9 suggest good reliability, and values exceeding 0.90 denote excellent reliability.

3. Results

3.1. Bayesian Model Comparisons

Bayesian Model Comparison of a null model (which included video series and observers as random effects), and a 'condition' model (which included the conditions EMG, ACC, and VIS as fixed effects) indicated there was "extreme" evidence in favour of the model including the factor of condition (BF_{10}= 11381). A grouped scatter plot demonstrates the effect of the different conditions (Fig. 2).

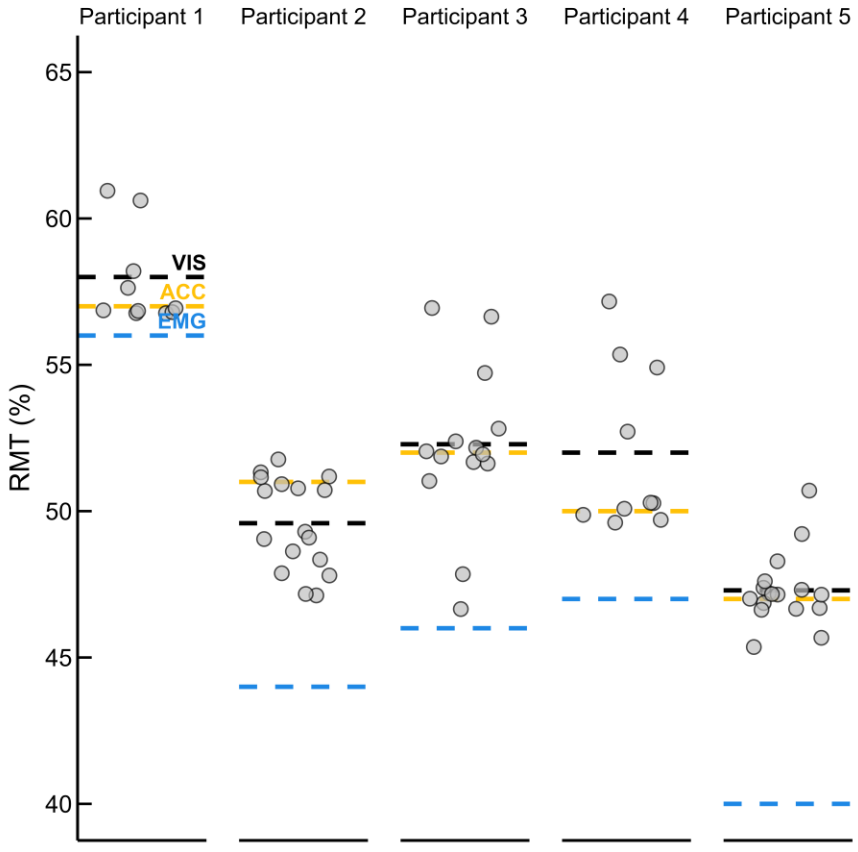


Figure 2. Individual Resting Motor Threshold (RMT) estimates by method and participant. Each panel shows data for one of the five participants from Group 1. Grey dots represent RMT estimates based on visual inspection responses from one of the

individual observers in Group 2. Dashed lines indicate the mean RMT obtained via each method: electromyography (EMG, blue), accelerometry (ACC, yellow), and visual inspection (VIS, black).

The Model Averaged Posterior Summary provides estimates for key variables (Table. 1). Notably, the RMT as estimated by EMG was lower than that for either ACC or VIS, with non-overlapping confidence intervals for these comparisons indicating there was a statistically meaningful difference when estimating the RMT using EMG compared to other techniques. By contrast, the overlapping 95% confidence intervals for ACC and VIS indicates their estimates are statistically indistinguishable within the model, suggesting no meaningful difference in estimates of the RMT exists between these two techniques. Furthermore, the positive estimates for ACC and VIS and the fact that the 95% credible intervals remain entirely above zero, suggesting a tendency to overestimate RMT when compared to EMG.

				95% Credible Interval	
	Level	**Mean**	**SD**	**Lower**	**Upper**
Intercept		50.1	2.1	45.6	54.4
Condition	**ACC**	1.3	0.6	0.1	2.4
	EMG	-3.0	0.6	-4.3	-1.8
	VIS	1.7	0.5	0.6	2.8

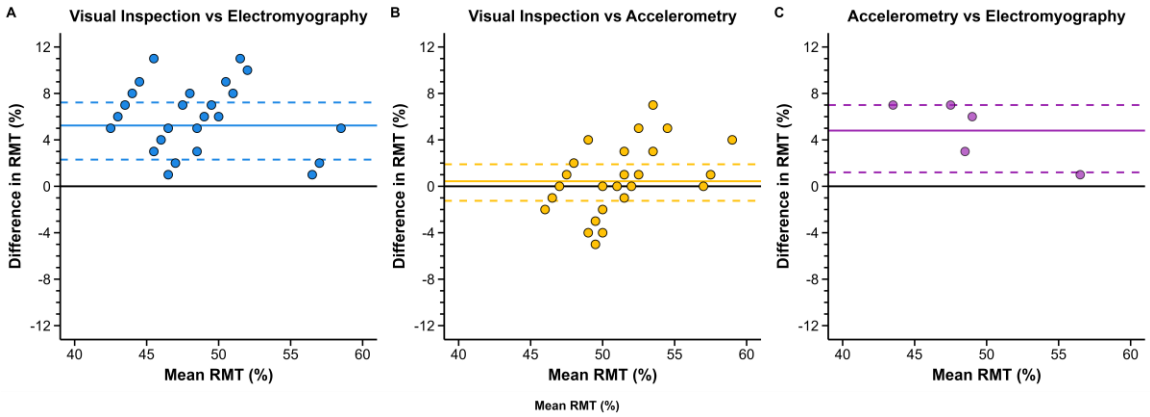
371 *Table 1: Model-averaged posterior summary statistics for the effects of condition on*
372 *Resting Motor Threshold (RMT). The intercept reflects the grand mean baseline RMT (in*
373 *% of maximum stimulator output), while condition estimates indicate the deviation*
374 *from the intercept for each technique: accelerometry (ACC), electromyography (EMG),*
375 *and visual inspection (VIS). Positive values represent an overestimation relative to the*
376 *baseline, and negative values represent an underestimation. Posterior means, standard*
377 *deviations (SD), and 95% credible intervals are reported.*

378 **3.2. Bland-Altman analysis**

379 When comparing EMG to VIS (Fig 3A), the analysis revealed a systematic bias, as the
380 95% confidence interval of the mean difference did not include zero, indicating that
381 VIS consistently overestimated RMT relative to EMG rather than fluctuating randomly
382 around it (mean=5.30, 95%CI=2.30-7.22) Additionally, the wide confidence interval
383 suggested substantial variability in the differences between VIS and EMG data.

384 By contrast, when comparing ACC to VIS (Fig 3B), the analysis indicated no systematic
385 bias, as the 95% confidence interval of the mean difference included zero
386 (mean=0.44, 95%CI=-1.25-1.90). However, despite this absence of bias, the broad
387 confidence interval suggested high variability across observers, indicating that while
388 VIS did not consistently over- or underestimate ACC, individual assessments varied
389 considerably between observers.

390 Finally, comparing EMG to ACC (Fig 3C), the analyses showed systematic bias, as the
391 95% confidence interval of the mean difference is higher than zero (mean=4.8, 95%CI
392 =1.20-7.00).



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394 *Figure 3: Bland-Altman plots comparing Visual Inspection with Electromyography (A),*
395 *Visual Inspection with Accelerometry (B) and Accelerometry with Electromyography*
396 *(C) as reference methods for resting motor threshold. The x-axis represents the mean*
397 *RMT (%) between the two methods, while the y-axis shows the difference in RMT (%)*
398 *between visual inspection and the reference method. Each dot represents an individual*
399 *measurement pair (though note that the data presented here are representative of a*
400 *bootstrap analysis with 10,000 resamples; due to the high density of data, numerous*
401 *data points overlap). The solid horizontal line indicates the mean bias, representing the*
402 *systematic difference between the methods, while the dashed lines denote the 95%*
403 *confidence interval. Each plot includes 10,000 data points from the resamples. Due to*
404 *the high density of data, numerous points overlap in regions, leading to visual clustering.*

405

3.3. Intraclass Correlation Coefficient Analyses

Summary statistics for the bootstrapped analysis are provided in Table 2.

For the bootstrap based ICC (2,1) analyses, when comparing VIS to EMG, the mean ICC value identified only 'acceptable' reliability, and the 95% confidence interval included responses ranging from 'unacceptable-to-good' (mean ICC=0.58, 95%CI=0.39-0.74), with the majority of resamples indicating only 'acceptable' reliability. By contrast, when comparing VIS to ACC, the mean ICC value identified 'good' reliability, with a 95% confidence interval including a range of responses spanning from 'acceptable-to-excellent' (mean ICC = 0.84, 95%CI=0.62-1.00) (Fig. 4).

To complement these results, ICC (2,k) values based on averaged ratings were also calculated. These showed "excellent" reliability for VIS vs ACC (point estimate = 0.980, 95% CI = 0.815–0.997) and "good" reliability for VIS vs EMG, though the 95% confidence interval indicated considerable variability, including responses ranging from 'unacceptable-to-excellent' (point estimate = 0.753, 95% CI = 0.132–0.973).

Finally, the ICC (2,1) comparison between EMG and ACC showed "acceptable" reliability, again with a wide 95% confidence interval, which included responses ranging from 'unacceptable-to-excellent' (point estimate= 0.587, 95%CI=0.108-0.943). No ICC (2,k) was calculated for this comparison due to the limited number of observations available for EMG and ACC.

	ICC (2,1)			ICC (2,k)		
	Mean Point Estimate	**95% CI**		**Point Estimate**	**95% CI**	
		Lower	**Upper**		**Lower**	**Upper**
VIS vs ACC	0.845	0.615	1.000	0.980	0.815	0.997
VIS vs EMG	0.580	0.389	0.748	0.753	0.132	0.973
ACC vs EMG	0.587	-0.108	0.943	N/A	N/A	N/A

Table 2: Intraclass Correlation Coefficient (ICC) values comparing Resting Motor Threshold (RMT) point estimates across methods. ICC (2,1) values reflect agreement between individual observer responses, while ICC (2,k) values reflect agreement based on average ratings per condition. Comparisons include visual inspection (VIS), accelerometry (ACC), and electromyography (EMG). Values are presented with their corresponding 95% confidence intervals. No ICC (2,k) was calculated for the ACC–EMG comparison as only one comparison was available per due to limited sample size.

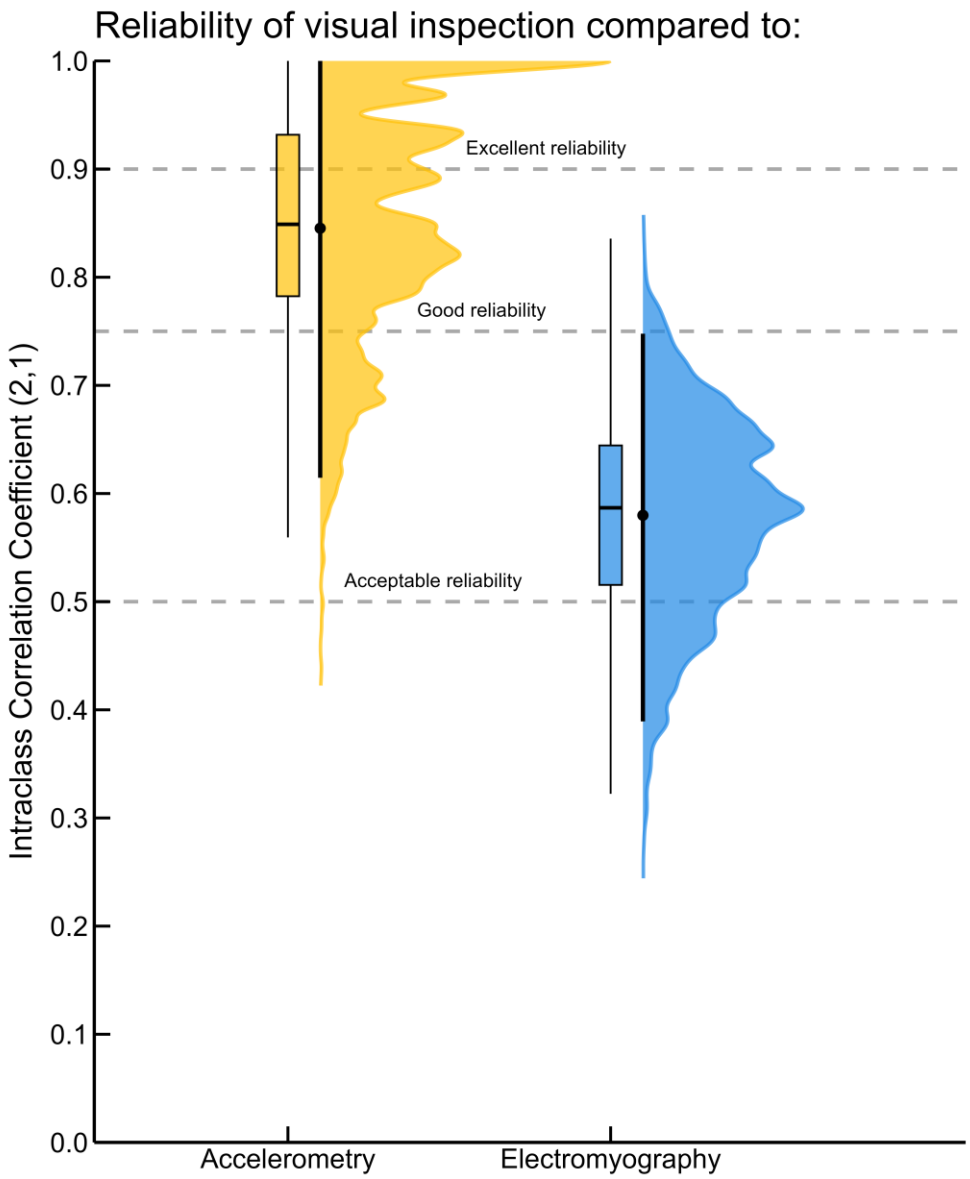

Figure 3 : Results of the bootstrap analysis demonstrating the distribution of Intraclass Correlation Coefficients (ICC 2,1) for visual inspection compared to accelerometry (Yellow) and electromyography (Blue). Violin plots represent the full distribution of ICC values with the dot representing the mean and vertical line the 95%CI, while box plots indicate the median, interquartile range, and overall dispersion. Each dashed line represents the reliability threshold based on Koo and Li (2016).

4. Discussion

The present study compared the resting motor threshold as estimated using electromyography, accelerometry, and visual inspection. A series of analyses indicated that while estimates of the resting motor threshold produced by accelerometry and visual inspection are generally similar, these techniques typically over-estimate the threshold as determined using electromyography. However, further analysis supported the view that accelerometry provides a more objective and quantifiable approach to determining the resting motor threshold than visual inspection, which varied considerably between observers. These findings support the view that electromyography remains the gold-standard for estimating the resting motor threshold, but in cases where the use of electromyography is not feasible, the objective and less variable nature of accelerometry provides a preferable alternative to visual inspection.

The present manuscript supports the view that estimation of the resting motor threshold using electromyography represents the 'gold standard'. Bayesian Model comparison and Bland-Altman analyses both indicated that visual inspection over-estimated the RMT when compared to the use of EMG. This overestimation is easily explained by the fundamental differences in what each method measures. EMG captures an intrinsic characteristic of the muscle—its electrical activity—providing a direct assessment of neuromuscular activation. In contrast, accelerometry and visual inspection rely on an extrinsic characteristic (i.e., overt movement of the finger). Muscle contraction (detectable by EMG) does not always result in displacement of the finger (as measured using ACC and VIS), and movement requires overcoming factors

462 such as inertia and frictional forces. These differences would explain why ACC and
463 VIS tend to overestimate RMT, as they may fail to detect subtle contractions that do
464 not produce sufficient movement.

465 These results are partially in line with the previous research that has compared the
466 use of visual inspection to EMG when estimating the resting motor threshold (43,44).
467 However, the protocols and findings of these previous studies remain subject to
468 discussion. Pridmore et al. (1998) (42) reported that motor thresholds determined
469 using EMG compared to visual inspection showed a difference of less than 10% in
470 percent of maximum stimulator output, suggesting a general consistency between
471 these approaches. However, they also found that in most cases, the threshold detected
472 through visual inspection was reached at *lower* intensities than with EMG. This
473 discrepancy raises questions about the comparability and sensitivity of these
474 methods in assessing cortical excitability, particularly regarding the potential
475 influence of methodological differences on threshold determination. Meanwhile,
476 Balslev et al. (2007) (45) employed a visual inspection protocol assessing the effects
477 of eight different coil orientations on the resting motor threshold as estimated using
478 EMG and visual inspection. While their results indicated that visual inspection
479 typically over-estimated the RMT as determined by EMG by approximately 2% of
480 MSO, as the comparison between these two methods was not the main focus of their
481 study, only data from four participants using a primarily repeated-measures design
482 (i.e. 8 different coil orientations were assessed for each participant) was collected.
483 This procedure may therefore have led to an under-estimation of differences between
484 the RMT as estimated using EMG compared to visual inspection. We also note that

485 the results of the present study are generally in line with the findings of our previous
486 research (49), in which we found that the RMT as estimated via EMG and ACC were
487 highly correlated, but that RMTs identified by EMG were generally lower than those
488 identified using accelerometry. An important consideration when interpreting our
489 findings is the pronounced measurement error inherent to VIS, which distinguishes
490 it from techniques such as EMG and accelerometry. When the same motor event is
491 recorded and analyzed using EMG or accelerometry, and the same detection
492 algorithm is applied, the outcome is reproducible, yielding a single, objective value
493 per trial. In contrast, VIS introduces considerable variability, as it relies on subjective
494 human judgment.

495 Further analyses considered whether accelerometry provided any benefits compared
496 to visual inspection. Here we note that while accelerometry provides a single
497 measurement that can be quantified objectively, visual inspection, by comparison,
498 remains a more subjective approach to identifying the Resting Motor Threshold.
499 Bland-Altmann analysis indicated that while the average RMT provided by visual
500 inspection matched well to that of estimates provided by EMG, there was
501 considerable variability around the mean (95%CI ranging from −4 to +5 MSO).
502 Similarly, our ICC bootstrapping analysis indicated that while on average VIS has
503 "good" reliability when compared to ACC, there was again a large disparity in the
504 95%CI, indicating that, in the best-case scenario, an observer (VIS) may provide a
505 value close to ACC, but in other cases, their estimate of the RMT could be far from it.

506 Given these findings, we propose that researchers who are not able to use EMG to
507 determine the RMT should prefer the use of accelerometry, rather than visual
508 inspection, wherever possible. Unlike VIS, which depends on individual observers
509 and introduces variability, accelerometry provides a single, consistent measurement
510 per trial, reducing subjectivity and improving measurement reliability. Nonetheless,
511 a limitation of the accelerometry is that it requires real-time (online) data processing
512 through a dedicated script.

513 However, several limitations should be acknowledged. First, the sample size for the
514 EMG and the accelerometry conditions was limited to five participants, which
515 constrains the generalizability of these findings and increases the risk of sampling
516 bias. We note, however, that the results of the comparisons between EMG and ACC in
517 the present study are in agreement with our previous work which has demonstrated
518 the same effects in a larger sample size (49). Second, a large proportion of the data
519 for visual inspection were collected in a remote, online experiment, which prevented
520 close monitoring of participant engagement. The inclusion of attention checks
521 therefore helped to maintain high quality data (i.e. approximately 1/3 of participants
522 were rejected based on this measure). Notably, no participants were rejected in the
523 in-person condition, suggesting that the physical presence of the experimenter likely
524 contributed to sustained engagement and compliance with the task. Third, the visual
525 inspection condition relied on a participant reviewing recorded videos, rather than
526 observing the stimulation in real time.

527 A further limitation is that the observers in the present study were relatively 'naive'
528 compared to experienced TMS experimenters. While training might reduce
529 variability, individual perceptual differences would likely persist, meaning that visual
530 inspection would still be less reliable than accelerometry. However, VIS could be
531 considered in studies using 'main' protocols delivered at submaximal RMT-based
532 intensities (e.g. 90% of RMT) to avoid safety issues (Rossi, Simone et al., 2009),

533 In addition, the recorded hands used for Group 1 were limited to a small sample
534 (n=5), which restricted the diversity of visible features such as gender, skin tone, and
535 hand or digit morphology. As a result, observer–actor mismatching may have
536 contributed, even in a small part, to the perceptual judgments of finger twitches.
537 While unlikely to account for the main effects observed, this highlights the need for
538 future studies to examine visual detection of MEP responses across more
539 demographically diverse samples.

540 Thus, visual inspection would still introduce variability to the measurement of the
541 resting motor threshold that accelerometry would remove. Additionally, the
542 convergence of our findings with previous research (e.g., our prior work on EMG and
543 ACC correspondence) further supports the reliability of the present data. In
544 particular, the strong agreement observed between EMG and ACC measurements
545 confirms the replicability of our previous results, and highlights the potential of using
546 an equation to convert the RMT as calculated by accelerometry to an estimated value
547 of the RMT as would be determined by EMG. Accelerometry therefore remains a
548 viable alternative when EMG is not available.

549 In conclusion, the present study indicates that EMG provides the most sensitive
550 approach to identifying the RMT, most likely due to its ability to measure the intrinsic
551 excitability of the corticospinal system. In situations where access to
552 electromyography is limited, researchers should consider using accelerometry in the
553 place of visual inspection, as accelerometry provides a more objective and reliable
554 approach.

555

5. Research data

The research data supporting the findings of this study are stored in an OSF (Open Science Framework) repository and are available upon reasonable request from the corresponding author at gautier.hamoline@uclouvain.be. Open public access is restricted to protect participant confidentiality in accordance with the guidelines of the approving ethics committee.

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7. Author Roles (CRediT)

Author	Conceptualization	Data curation	Formal analysis	Funding acquisition	Investigation	Methodology	Project administration	Resources	Software	Supervision	Validation	Visualization	Writing – original draft	Writing - review and editing
Hamoline Gautier	x	x	x		x	x	x	x	x	x	x	x	x	x
Antoine Lunardi		x			x								x	
Marcos Moreno-Verdu			x						x			x		x
Baptiste M Waltzing		x												x
Elise E Van Caenegem		x												x
Siobhan MacAteer									x					x
Robert M Hardwick	x			x		x	x		x	x	x		x	x

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