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Day-to-day variability in motor threshold during rTMS treatment for depression: Clinical implications

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ABSTRACT

Background: When repetitive transcranial magnetic stimulation (rTMS) is used to treat medication refractory depression, the treatment pulse intensity is individualized according to motor threshold (MT). This measure is often acquired only on the first day of treatment, as per the protocol currently approved by Food and Drug Administration.
Objective: Here, we aimed to assess daily MT variability across an rTMS treatment course and simulate the effects of different schedules of MT assessment on treatment intensity.
Methods: We conducted a naturalistic retrospective study with 374 patients from a therapeutic rTMS program for depression that measures MT daily.
Results: For each patient, in almost half the TMS sessions, MT varied on average more than 5% as compared to the baseline MT acquired in the first treatment day. Such variability was only minimally impacted by having different TMS technicians acquiring MT in different days. In a smaller cohort of healthy individuals, we confirmed that the motor hotspot localization method, a critical step for accurate MT assessment, was stable in different days, arguing that daily MT variability reflects physiological variability, rather than an artifact of measurement error. Finally, in simulations of the effect of one-time MT measurement, we found that half of sessions would have been 5% or more above or below target intensity, with almost 5% of sessions 25% above target intensity. The simulated effects of weekly MT measurements were significantly improved.
Conclusions: In conclusion, MT varies significantly across days, not fully dependent on methods of MT acquisition. This finding may have important implications for therapeutic rTMS practice regarding safety and suggests that regular MT assessments, daily or at least weekly, would ameliorate the effect.

1. Introduction

Major depressive disorder (MDD) is a common psychiatric disorder, affecting more than 300 million people worldwide, and accounting for 10% of all years lived with disability globally [1]. Importantly, a significant proportion of patients do not respond to pharmacological treatments [2–4]. Repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex

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(DLPFC) is a therapeutic strategy for treatment-resistant depression (TRD) [5], with evidence for long-lasting efficacy among patients that respond to this treatment [6]. For those who do not respond to rTMS, patient-related factors [7], as well as treatment-related factors such as the location of stimulus application [8,9], may account for reduced neuromodulatory and clinical effects [10,11]. Treatment dose, which is individualized according to resting motor threshold (MT), could also influence antidepressant response [12,13].

Motor threshold is the intensity of motor cortex stimulation that produces at least 50% successful motor responses in the contralateral hand in a series of consecutive stimuli [14]. In currently approved protocols, including by Food and Drug Administration (FDA), for therapeutic rTMS in TRD [12,15], stimulation dose is individualized for each patient according to the MT measured on the first day of treatment, and then kept constant across days. However, it is unclear if variability of MT across days is significant, and if this contributes toward treatment efficacy and/or safety. In fact, MT varies according to different factors such as changes in sleep [16], diet [17], time-of-day [18], and medical conditions [19], among others [20]. If MT does in fact vary across a course of TMS for depression treatment, protocols with infrequent MT determination could provide ineffective stimulation due to "underdosing" in sessions where MT has increased, or raise safety concerns due to "overdosing" in sessions where MT has decreased [21].

Here, using retrospective data from a TMS clinical center where MT is acquired and recorded daily, we describe daily MT variability across treatment courses. Using these data, we also simulate the potential for over or understimulation in each therapeutic session if MT is measured at a less-than-daily frequency. In a separate smaller group of healthy individuals, we further test the stability of methods for MT acquisition, namely regarding motor hotspot (M1) localization, to determine whether this could be a major source of variability.

2. Methods

2.1. Study design

We conducted a naturalistic retrospective study, with data from patients treated at the Berenson-Allen Center for Noninvasive Brain Stimulation (BA-CNBS) from 2000 to 2019, in compliance with local Internal Review Board approval for publication of clinical data.

2.2. Population

Adult patients treated with DLPFC rTMS for depression, using either Magstim or Neuronetics devices, were eligible. Depression was considered as any major depressive episode in the context of either MDD or bipolar disorder type II, according to the Diagnostic and Statistical Manual of Mental Disorders [22–24]. Patients were excluded if MT for the first rTMS session could not be retrieved ($n = 16$), if treatment device was changed during the treatment course ($n = 5$), if less that 10 rTMS sessions were conducted in total ($n = 9$), or if average interval between consecutive sessions was 2.5 days or more ($N = 8$). Furthermore, for analyses where treatment response or remission was considered, patients with baseline Beck Depression Inventory-II (BDI-II) [25] score less than 14 [26], were also excluded ($N = 24$).

2.3. Variables

Age, gender, baseline and follow-up severity scores (BDI-II and 17 item-Hamilton Depression Rating Scale – HAM-D-17) [27], daily stimulation parameters (device, coil, stimulation-side, stimulation intensity, frequency, total number of pulses per session), medication and MT were extracted from the electronic clinical database, whenever available. There was also variability in TMS technician, and therefore, this factor was extracted whenever available.

MT was determined from the motor hotspot (M1) using the visual method in accordance with the available clinical recommendations [28]. In the application of these methods, trained TMS technicians started by locating the cranial vertex at the cross-section between the *nasion-inion* mid-sagittal line and the inter-tragus line. The center of the stimulating coil was then placed on a point 5 cm from the vertex on the inter-tragus line, which was the starting point for functional location of M1. With the TMS output set to a low intensity, stimulation pulses were delivered, and the contralateral hand inspected visually for muscle twitching, with intensity increased after at least 3 stimuli without a response. Once a visible twitch was observed in the target muscle-group, i.e., *abductor pollicis brevis* or *first digit interosseous*, intensity was kept constant to compare muscle twitching responses obtained from stimuli applied to other locations, namely 0.5 cm and 1 cm anterior, posterior, medial and lateral to the original stimulation point. The location eliciting, most consistently, the largest muscle twitch of the target muscles was thus defined as the M1. The treatment target in the dorsolateral prefrontal cortex (DLPFC) was then identified as the point 5–5.5 cm anteriorly to M1, on the corresponding parasagittal line, according to the available clinical recommendations [28].

Once M1 location was defined, the resting MT for M1 was determined according to the visual method, one of the strategies supported in the consensus recommendations for clinical application of rTMS in depression treatment [28], systematized according to our center experience and best clinical practice. In this process, starting with the supra-threshold stimulation intensity used to locate M1, 10 pulses, spaced 6–10 s apart were delivered at M1 while visually inspecting the target muscle for muscle twitching. Intensity was then reduced by 2% in sequential steps, until less than 5 out of 10 TMS trials elicited a visible muscle twitch (i.e., below threshold). Finally, intensity was increased by 1% and, if at least 5 of 10 TMS trials elicited a visible muscle twitch, that intensity was defined as the motor threshold. Biphasic pulse shape was used in MT determination and during the treatment sessions.

2.4. Data analysis

All data were analyzed using StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC. Clinical response to rTMS was calculated as the percent reduction of BDI-II depression severity scores at the last measurement, relative to baseline (BDI-response). Normalized MT (nMT) was calculated in each patient for all treatment days after the first ($day_{2\text{-}30}$), as the ratio of that day MT relative to MT on the first day ($nMT_{2\text{-}30} = MT_{2\text{-}30}/MT_1$). In a simulation analysis, the actual treatment stimulation intensity for the first day (aSI_1) was 120% MT and considered as the treatment stimulation intensity that would have been used in the following days if MT had been measured only on the first day. The simulated stimulation intensity (sSI) for the following days, sSI was calculated according to a ratio of aSI_1 and the MT measured on the following days: $[sSI_X=(aSI_1/MT_X)*100]$% of MT. Similar computations were performed to simulate sSI if MT had been measured weekly ($[^{w1}sSI_X$ to $^{w6}sSI_X]$% of MT), using aSI_1, aSI_6, aSI_{11}, aSI_{16}, aSI_{21} and aSI_{26} for weeks 1, 2, 3, 4, 5 and 6, respectively. Please see Fig. S1 for further details on data analysis calculations. The percentage of sessions per patient in determined nMT intervals (<0.75, [0.75; 0.85[, [0.85; 0.95[, [0.95; 1.05[, [1.05; 1.15[, [1.15; 1.25[and ≥1.25) as well as sSI intervals (<95%, [95; 105%[, [105; 115%[, [115; 125%[, [125; 135%[, [135; 145%[and ≥145%) was then calculated, as was

the number of patients within given ranges of number of sessions (1–3, 4–6, 7–9 and ≥10) in each of the sSI intervals.

Data for continuous measurements (age, number of sessions, MT, BDI-II, HAM-D-17, BDI-response, HAM-response, nMT, sSI, percent sessions in determined nMT or sSI intervals) are presented as mean ± standard error of the mean (SEM) and analyzed, as appropriate, using unpaired two sample t-tests, analysis of variance (ANOVA) and longitudinal mixed effects regression analyses. Binary outcomes (gender, stimulation side and patients with a given number of sessions within each of the sSI intervals) are presented as percentage of patients (%) and analyzed using Chi-square tests. In a sub-cohort of individuals (N = 89) for whom information of identity of TMS technician in each day was available, we calculated, for each patient, mean consecutive nMT variability, separately for consecutive rTMS sessions performed by the same and by a different TMS technician, and are also presented as mean ± SEM. Paired t-tests were used to compare nMT variability across patients between same vs. different TMS technician.

2.5. Reliability of M1 localization

In addition to the retrospective study described above, a small cohort of healthy individuals were also recruited prospectively, at the Champalimaud Center for the Unknown (CCU), to assess test-retest reliability of M1 localization procedures and determine whether this could contribute to MT variability. The protocol consisted in determination of left M1 location in two separate days and was performed according to protocol approved by the local ethics committee, in accordance with the Declaration of Helsinki. Ten right-handed healthy adults, ages 22-50 years-old, five of whom male, were recruited. Previously acquired T1-weighted MRI brain scans from each participant were loaded into a Visor 2 Neuronavigation System (ANT Neuro, Enschede, Netherlands) for neuronavigation-based measurements. In each session a trained TMS technician located M1 according to the methodology described above and saved stereotaxic coordinates for later offline analysis. The TMS technician was the same for all research subjects in both sessions and was blinded to neuronavigation images and coordinates. The anatomical images and associated target coordinates from the neuronavigation system were co-registered into a common anatomical space (MNI152; http://nist.mni.mcgill.ca/) using a composition of 3D-rigid and affine transformations in 3D-Slicer Software. The procedure was repeated in two sessions in order to assess between-session reliability. Scalp and cortical coordinates for left M1, as well as the within-subject distance between the same target measured in different sessions, were acquired offline using Visor 2 tools. To assess between-session reliability of left M1 localization we first computed the absolute scalp distance between left M1 "hotspots" in the two sessions and calculated the respective coefficient of variation (CV=SD/Mean*100). Furthermore, we computed the intraclass coefficient (ICC), absolute agreement, using a two-way mixed-effects model [29] between the two sessions. We extracted the distance of each M1 coordinate to the origin of the coordinates system (0, 0, 0), and compared the two distances for each participant. We also computed CVs for such distances in each session (CV_1; CV_2).

3. Results

Clinical and demographic characteristics of the retrospective sample of depressed patients treated with rTMS are presented in Table 1, with comparisons of baseline characteristics for patients treated with each of the two main devices used in this treatment center. In patients where medication was systematically reported (N = 175), we found that 96.0% were on any type of medication: Benzodiazepines – 50.9%; Antipsychotics – 43.4%; Anticonvulsants – 34.3%; Selective Serotonin Reuptake Inhibitors – 34.3%; Serotonin and Norepinephrine Reuptake Inhibitors – 32.0%; Bupropion – 14.9%; Lithium – 14.3%. Raw MT values were not directly comparable across devices due to different scales. However, nMT could be calculated and compared for the two devices. We found that nMT did not change across the course of therapy ($-2.5*10^{-4}\pm1.5*10^{-4}$, p = 0.1; Fig. 1A) and that there was no significant effect of gender, age, side of stimulation, year of treatment or baseline BDI on nMT ($-2.0*10^{-4}\pm1.7*10^{-4}$, p = 0.2). No differences in nMT variability across time were found according to responder status (responder vs. non-responder; Fig. 1B) or stimulation device (Magstim vs. Neuronetics; Fig. S2). On analyses of per-patient summary data across treatment days, MT varied 5% or more above or below the first MT, on average, in almost half of the sessions per patient (42.0 ± 0.4%), with more extreme variations (≥25% above or below the first MT) in, on average, 1.3 ± 0.2% of sessions per patient (Fig. 1C). Also, on the per-patient analyses, while the aforementioned variations were similar among the two stimulation devices (Fig. S3) and between early (2000–2007) and later (2008–2019) years of treatment (Fig. S4), there were differences between responders and non-responders, suggesting a greater tendency for MT reduction across sessions in responders (Fig. 1D). These analyses were repeated using raw MT values, separately for Magstim and Neuronetics and, while the differences between responders and non-responders were not reproduced, possibly due to reduced statistical power, nMT variability across time was consistent in both sub-cohorts.

To explore factors that could influence day-to-day variability in MT measurements, in patients for whom such data was available, we compared mean nMT variability in consecutive treatment days, according to presence vs. absence of variation in TMS technician. For patients for whom TMS technician was systematically recorded on a daily basis (N = 89), per patient mean consecutive day nMT variability was, on average, slightly higher when the technician changed, compared to when it was performed by the same operator (3.6 ± 0.2% vs. 2.9 ± 0.2% respectively; p = 0.0001, paired *t*-test). Furthermore, when comparing the performance of individual technicians regarding mean nMT variability in consecutive days across patients i.e., the session-to-session variability for a specific technician, the amount of variability in MT did not differ among technicians, with mean nMT variability across technicians of 3.1 ± 0.2% (p = 0.1, linear regression).

Given the evidence that daily variability in MT was only partially explained by variation in the TMS technician, and also the evidence for equivalent performance between TMS technicians regarding consecutive nMT variability, a major source of MT variability could be due to motor hotspot location variability. In a separate prospective cohort (Table 2), we assessed between day stability in M1 localization, a potential source of MT daily variability. This was done with a single rater i.e., TMS technician, using the same method as that described for the retrospective patient cohort, across TMS sessions, in two separate days. Noteworthy, the single rater had similar left-side nMT variability (3.0 ± 0.8%) relative to the aforementioned technicians, when assessing MT in similar circumstances. When comparing M1 location identified in each of the two experimental sessions using a neuronavigation system (please see methods for details), we found that median scalp distance between targets was 8.2 mm (IQR = 5.8–8.9; CV = 44.8%), while the intraclass coefficient correlation (ICC) for cortical distances of each M1 location to the origin of the coordinate system (0, 0, 0) was high ($ICC_{Average}$ = 0.8, 95% CI 0.4–1.0; $ICC_{Individual}$ = 0.7, 95% CI 0.3–0.9; F [9,9] = 6.4; p = 0.006) and the respective CVs for each session extremely low (CV_1 = 3.9%; CV_2 = 5.2%), confirming that M1 was consistently found within less that 1 cm of the previous location.

Table 1
Clinical and demographic characteristics of the study sample.

Characteristic	Total Sample (N = 374)	Magstim[a] vs. Neuronetics[b]		
		Magstim (N = 282)	Neuronetics (N = 92)	P value
	Mean ± SEM or %	Mean ± SEM or %	Mean ± SEM or %	
Age	50.0 ± 0.8	49.1 ± 1.0	52.6 ± 1.5	n.s.
Gender (% Female)	59.0	58.8	59.8	n.s.
Stimulation Side (% Left)	89.4	91.4	83.3	0.03
Nº of sessions	24.3 ± 0.3	24.1 ± 0.4	24.8 ± 0.6	n.s.
Stimulation Intensity (% of MT)	114.6 ± 0.3	112.5 ± 0.4	118.6 ± 0.4	<0.001
Frequency (Hz)	13.5 ± 0.4	13.8 ± 0.4	13.0 ± 0.7	n.s.
Total Number of Pulses	2218.2 ± 40.4	2208.3 ± 47.8	2242.4 ± 76.1	n.s.
Baseline MT	N.A.	64.1 ± 0.7	1.1 ± 0.02	N.A.
Mean MT	N.A.	63.4 ± 0.7	1.1 ± 0.02	N.A.
Mean nMT	1.0 ± 0.003	1.0 ± 0.004	1.0 ± 0.007	n.s.
Baseline BDI	31.9 ± 0.6	32.1 ± 0.6	31.4 ± 1.2	n.s.
BDI Response (% improvement)	38.4 ± 1.8	38.2 ± 2.0	39.2 ± 3.7	n.s.
BDI Treatment Response[c]	42.0%	41.1%	44.7%	n.s.
BDI Treatment Remission[d]	32.0%	31.3%	34.1%	n.s.
Baseline HAMD	22.1 ± 0.4	22.1 ± 0.4	22.0 ± 0.7	n.s.
HAMD Response (% improvement)	39.8 ± 2.1	37.7 ± 2.4	46.1 ± 4.2	n.s.

BDI – Beck Depression Inventory; HAMD – Hamilton Depression Rating Scale; MT – Motor Threshold; N – number of subjects; Nº – Number; N.A. – Non-Applicable; n.s. – not significant; nMT – Normalized Motor Threshold; SEM – Standard Error of the Mean.
[a] Magstim D70 Air Cooled coil was used in 279 patients and Brainsway H1 coil was used in 3 patients.
[b] Neuronetics Precision Pulse TMS coil was used in all patients.
[c] Response criteria were defined as a reduction of at least 50% from baseline.
[d] Remission criteria were defined as a final BDI score of ≤11.

To assess the potential clinical relevance of our findings, we returned to the data from the retrospective sample of depressed patients described above and simulated the effects on stimulation intensity of measuring MT only once at the beginning of the treatment or once every week. For that, we calculated stimulation intensity relative to baseline MT, modeling the effects of measuring MT only once per course of therapy, once weekly, or daily MT measures, with the simulated stimulation intensity (sSI) relative to the actual measured MT for that day (see methods for details). In fact, if MT had been measured only once per course of therapy, as is in most protocols cleared by the FDA, our simulation shows that, on average, half of the treatment sessions per patient would have been performed 5% or more above or below the recommended stimulation intensity (120% MT, i.e., 115–125% MT) [28]. However, the simulation shows significant advantages for measuring MT weekly with, on average, only one-third of sessions performed outside of the recommended intensity window in each patient and, specifically, a lower proportion of sessions being performed at high intensities (Fig. 2; see also Figs. S5A–D). Importantly, when data were considered according to number of sessions per patient, rather than proportion, we found that the majority of patients would have been treated in the recommended interval (115–125%) for 10 or more sessions both if MT was determined once or weekly (61% and 86% of patients, respectively). On the other hand, 9% and 4% of patients, respectively, would have been treated with a very high stimulation intensity (≥145% MT) in at least 1 session (see Table S1 for further details).

4. Discussion

To our knowledge, this is the first study reporting daily MT variability across rTMS depression treatments, allowing for an estimate of the effects of different intervals of MT measurement. Although, on average, MT decreased slightly (but not significantly) across days, it was 5% or more above or below the first MT, on average in almost half of the sessions per patient. Thus, while our results are consistent with prior findings of only minor MT change across days at a group level [30,31], they demonstrate significant day-to-day MT variability, with potential clinical relevance in terms of both efficacy and safety, that should be explored in future studies, designed to evaluate the impact of different MT assessment schedules. Unfortunately, few studies have assessed the relationship between MT and side effects, or efficacy, and the results have been inconclusive [31–36].

Different methods of MT estimation, such as Rossini–Rothwell, parameter estimation by sequential testing (PEST) or Bayesian inference PEST variant, have been described as potentially improving MT intra- and inter-session reliability, while decreasing the application time [37,38]. Nevertheless, such methods have been mostly used in research setting, using electromyography (EMG) rather than visual estimation, which was the method used in our study. In the clinical context, the visual method is advocated and widely practiced for ease of use and cost-effectiveness [28]. Because our study aims to inform current clinical practice, EMG-based methods were not considered, and MT variability could be potentially impacted by TMS operators. However, while MT variability may be explained to a small extent by between-technician differences or variability in M1 localization between sessions, our data suggest other physiological factors are major contributors to such variability. In fact, the variability in MT was only slightly larger when the technician varied across consecutive days, even if this small effect (0.7 ± 0.2%) was significant with this large database. Furthermore, clinical standard M1 localization method was found to be highly reproducible [39]. Hence, MT variability is unlikely to be completely dependent on methodologic constraints and could be reduced but not eliminated if recommended TMS-related procedures, including TMS technician training, are followed. To this end, the training recommendations endorsed by the International Federation of Clinical Neurophysiology provide valuable guidelines [40]. Individual neurophysiologic changes across treatment days likely contribute significantly to the observed MT variability. While such variability could result from somewhat unpredictable factors such as sleep [16], diet [17] or other factors [20], there are also suggestions of some degree of predictably related to improvement from depression [34]. In fact, we found that rTMS responders had more sessions with MT lower than on the first day, suggestive of increasing cortical excitability, when compared to non-responders. This finding is in support of the theory that patients diagnosed with

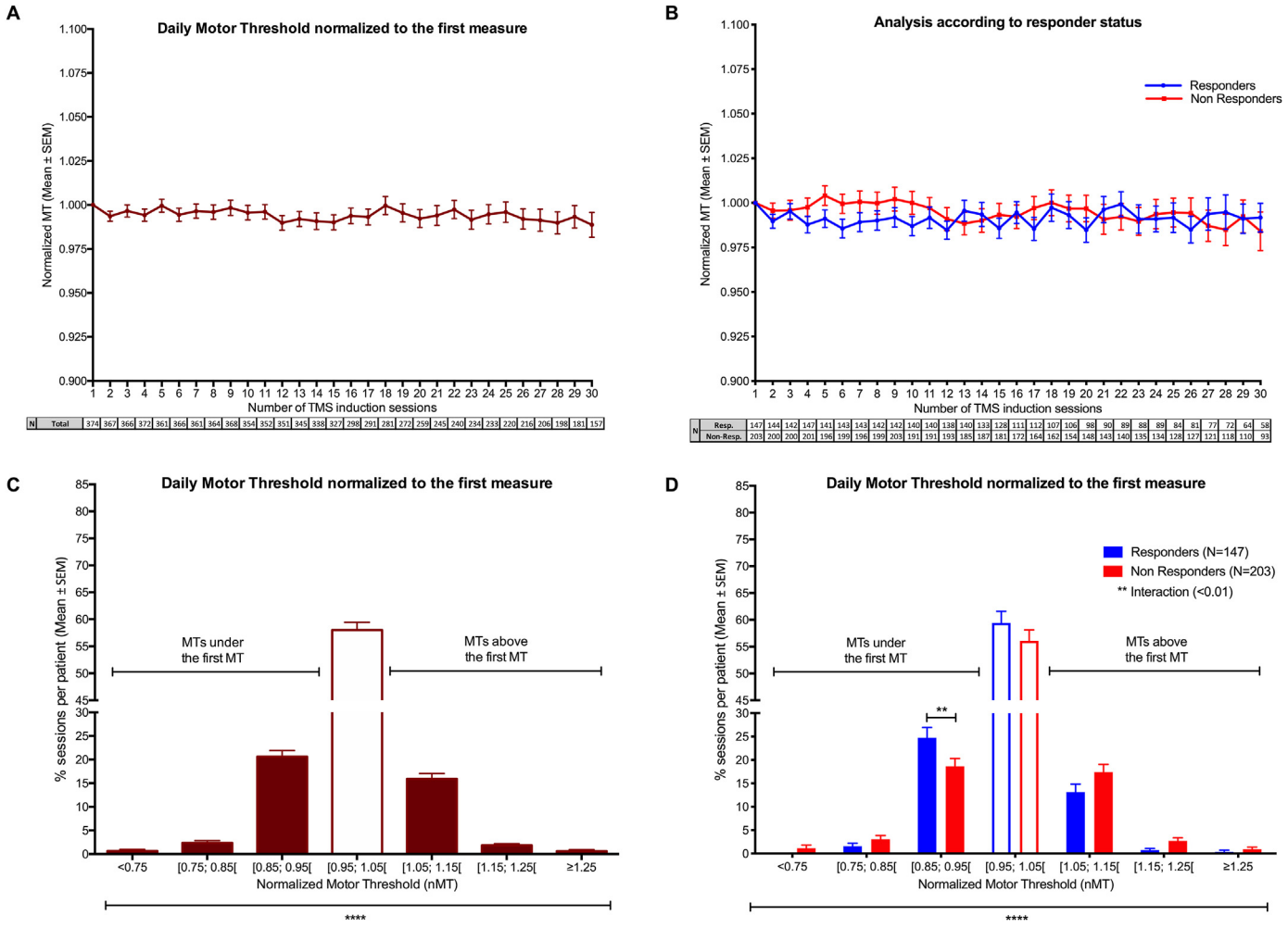

Fig. 1. Normalized Motor Threshold.
(A) When analyzed using longitudinal regression, daily normalized motor threshold (nMT) was found to decrease slightly, and close to significance, across days in the entire sample (ß = $-2.5*10^{-4}$±$1.5*10^{-4}$, p = 0.1). This effect was not significant (ß = $-2.0*10^{-4}$±$1.7*10^{-4}$, p = 0.2) when adjusting for other variables (gender, age, side of stimulation, year of treatment and baseline BDI, that were not significantly related to nMT – data not shown) in a longitudinal mixed effects regression model. In this plot, N is the number of patients with MT available for a specific session; while N mostly drops across time due to attrition, there are increases in some instances due to reductions in missing values. (**B**) In a longitudinal mixed effects regression model considering a possible association between nMT and treatment response status, while the decrease of nMT across days was close to significance (ß = $-2.4*10^{-4}$±$1.6*10^{-4}$, p = 0.1) there was no effect in the comparison between responders and non-responders (ß = $-8.1*10^{-3}$±$6.7*10^{-3}$, p = 0.2). These findings did not change significantly in other models, when adjusting for other variables (gender, age, side of stimulation, year of treatment and baseline BDI); however, while findings did not change significantly when including an interaction term for response status and days across the whole treatment, we found an interaction between these two variables in the second third of treatment course, i.e. from 11th – 20th sessions, revealing a tendency for responders to have a greater decrease than non-responders across this period (ß = $-1.5*10^{-3}$±$7.5*10^{-4}$, p = 0.04); regardless, post-hoc t-tests did not reveal differences in specific sessions. **(C)** The percentage of sessions per patient within defined nMT intervals (<0.75, [0.75; 0.85[, [0.85; 0.95[, [0.95; 1.05[, [1.05; 1.15[, [1.15; 1.25[and ≥1.25) varies significantly across such intervals ($F_{(6,\ 2238)} = 471.2$, $p < 0.00001$; repeated measures one-way ANOVA). **(D)** In analyses according to treatment response, while differences between nMT intervals were conserved ($F_{(6,\ 2088)} = 422.7$, $p < 0.00001$), treatment response did not impact nMT ($F_{(1,\ 348)} = 0.03$, $p = 0.9$) but interaction between the two factors was significant ($F_{(6,\ 2088)} = 3.0$, $p < 0.01$; repeated-measures two-way ANOVA). **p < 0.01, ****p < 0.0001, post-hoc Bonferroni corrected t-tests.

Table 2
Characteristics of subjects recruited for motor hotspot intra-rater reliability sessions.

Subject	Gender	Age (y)	Dominant Hand	Intersession Distance in Left M1 (mm)
1	Male	26	Right	6.81
2	Female	28	Right	5.58
3	Female	22	Right	7.79
4	Male	40	Right	4.67
5	Male	32	Right	8.60
6	Male	24	Right	5.76
7	Female	26	Right	10.47
8	Male	50	Right	8.54
9	Female	28	Right	18.16
10	Female	36	Right	8.90

M1 – Motor Hotspot; mm – millimeters; y – years.

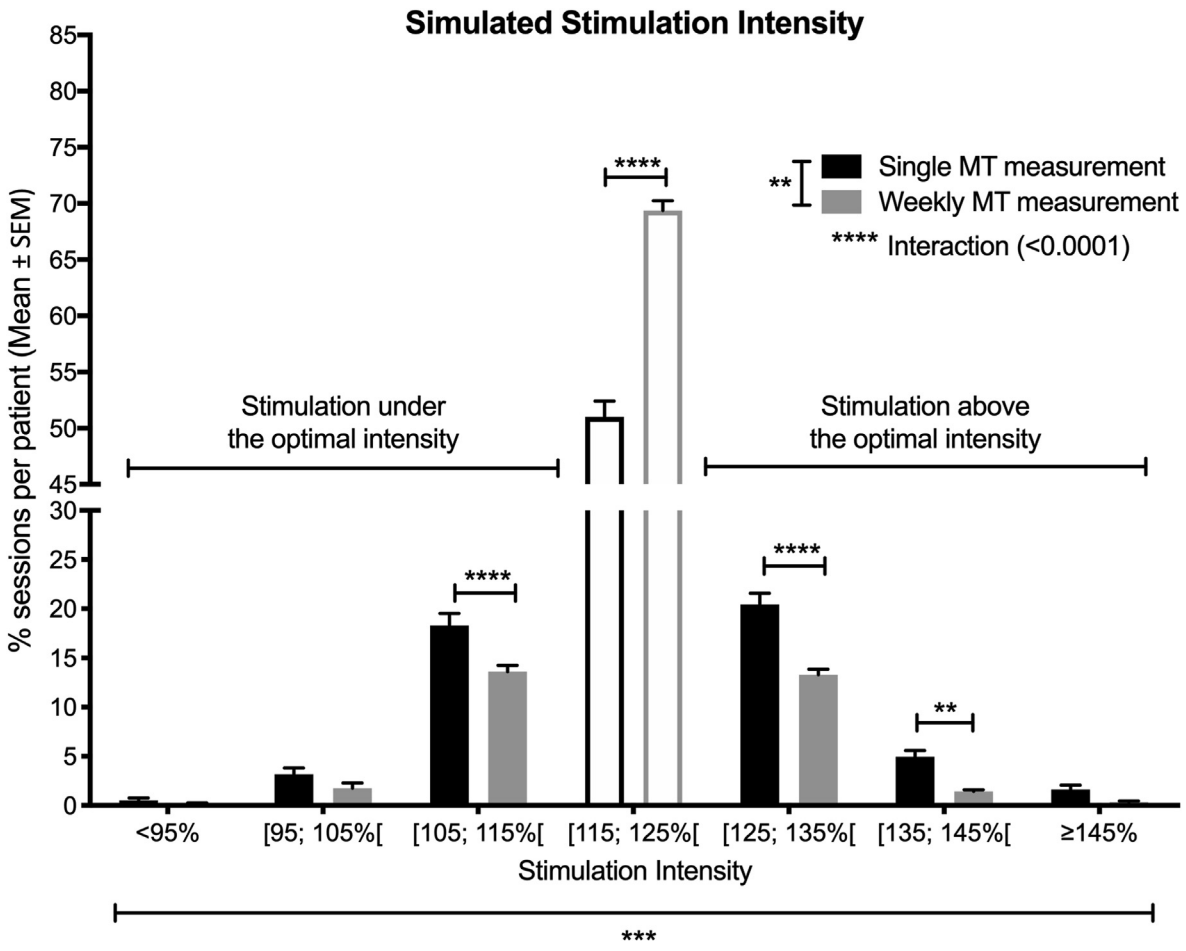

Fig. 2. Simulated Stimulation Intensity.
Simulated stimulation intensity (sSI) if MT had been measured only on the first session of treatment, or once at the start of each week, was calculated as defined in the methods section. As expected the percentage of sessions per patient within defined sSI intervals (<95%, [95; 105%[, [105; 115%[, [115; 125%[, [125; 135%[, [135; 145%[and ≥145%) was significantly different between sSI intervals ($F_{(6, 4476)} = 1426.4$, $p < 0.00001$). Furthermore, there was on one hand a significant difference according to frequency of MT measurement ($F_{(1, 746)} = 6.8$, $p = 0.01$), but also the two factors interacted significantly ($F_{(6, 4476)} = 55.1$, $p < 0.00001$; two-way ANOVA), revealing a clear advantage for weekly relative to single MT measurement. **p < 0.01, ****p < 0.0001, post-hoc Bonferroni corrected t-tests.

MDD have impaired cortical neuroplasticity and that is improved after successful antidepressant treatment [41]. Nevertheless, future studies assessing MT should be designed to systematically explore such hypothesis. Interestingly, the sustained effect of TMS in cortical excitability might be occurring at the synaptic level, where increasing synaptic noise has been hypothesized to impact neurons excitability and plasticity [42].

Our results are of particular relevance, and provide guidance, regarding the frequency of MT assessment across rTMS treatment of depression. According to our findings, it is reasonable to expect that, when MT is assessed only once in the beginning of treatment, 9% of patients will have at least one TMS session performed at very high intensity (≥145% MT), potentially posing important safety concerns. Weekly MT measurement is clearly advantageous when compared to a single MT measurement, leading to a reduction of the risk of overstimulation, since only 4% of patients would have been exposed to significant overstimulation in one session. Additionally, with MT weekly assessments only one-third of the sessions would have been performed outside the recommended parameters, while with a single MT assessment almost half are expected to be performed out of the recommended parameters. Importantly, one TMS manufacturer has also supported that weekly measurements of MT should be performed, and non-compliance of such MT assessment schedule has been recognized as a likely culprit for seizures [43,44].

Our results should nevertheless be interpreted considering the limitations of the study design. First, this study was mainly supported by a naturalistic cohort, hence no sham rTMS stimulation was used. Accordingly, MT variability could be due to natural neurophysiologic changes, the cumulative effects of rTMS, or other factors. Additionally, while the absence of a controlled environment may be considered an important caveat, we believe this can also be viewed as a strength. Previous evidence for MT stability have been extracted from highly controlled settings [31]. Such clinical research environments include highly selected patients' populations, often leading to an important impact on external validity and generalizability [45]. Hence, a naturalistic study design will more closely reflect MT variability in the more general patient population. Second, we have used a retrospective design, relying on records from a broad time range, where incomplete information is expected. Nevertheless, analyzing data from a wide range period is more likely to increase the probability of non-differential information/misclassification bias, favoring the null hypothesis instead of supporting a consistent significant result [46]. However, in order to address this potential limitation, we have used different strategies to focus on the most accurately reported data, such as

excluding patients who had no information about the first rTMS session, had changed device during the treatment course, had less 10 rTMS sessions or had infrequent MT assessments. Additionally, we have performed a number of sensitivity analyses, which included further analysis of data from a sub-population where additional information, namely identity of TMS technician, was available, as well as collecting a prospective sample to assess the reliability of the procedures that were used to determine MT in the retrospective cohort. We used MT as defined by visual observation, which while accepted and broadly used in clinical practice [28,38,47–51], may be less accurate that EMG-based determinations and thus may introduce greater variability. Finally, potentially important confounders for MT variability were not systematically collected, hence not considered, such as medication, smoking status, coffee and alcohol consumption, sleep duration, psychiatric and non-psychiatric co-morbidities, among others. While we believe this should be definitely considered in future prospective studies, their impact on MT variability is already known [16–18,20]. In fact, the impact of these potential confounders actually supports our conclusions, stressing the importance of measuring MT more often, especially in the presence of these MT variability moderators. Thus, we believe that our results, while speculative and limited by our study design and their associated constraints, generate important questions regarding both safety and efficacy of treatment based on the frequency of MT measurements. Such data may inform future studies comparing patients, or TMS Clinical programs, with different MT assessment scheduling strategies, in prospective designs.

5. Conclusion

In conclusion, we found that MT varies significantly on a daily basis within patients receiving rTMS treatment for depression. This variability may raise concerns about safety and efficacy of treatment when MT is only measured once at the start of therapy. The effect of MT variability could be explored in future studies designed to evaluate the impact of different MT assessment schedules in these clinical outcomes, though it would be challenging and naturalistic assessments like those presented here can help inform the field. These findings highlight the need for revisiting current rTMS protocols [31], exploring the implementation of more frequent MT assessments, such that stimulation is delivered consistently within the recommended intensity. Since it is unlikely that randomized-control-trials will be performed to demonstrate the effects of different MT determination frequencies on efficacy and side-effects, we believe that the evidence presented here support that more regular, daily or at least weekly, MT determination in clinical rTMS programs may be considered, to avoid moderate-to-severe under or overstimulation across a course of therapy.

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GC, AJO-M, AS, AP-L and DP conceived and designed the work; GC, AJO-M, CP, FFV, DRS, and CS acquired the data; GC, AJO-M and DP analyzed and interpreted data; GC, AJO-M and DP drafted the manuscript, that was critically revised by the remaining authors for important intellectual content. All authors approved the final version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. DP had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Order of co-first authors was decided by consensus.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2021.07.013.

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