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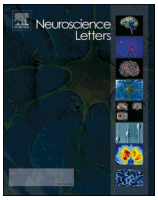

Research article

Comparison between conventional and neuronavigated strategies to assess corticospinal responsiveness in unfatigued and fatigued knee-extensor muscles

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ABSTRACT

In studying neuromuscular fatigability, researchers commonly use functional criteria to position and hold the transcranial magnetic stimulation (TMS) coil during testing sessions. This could influence the magnitude of corticospinal excitability and inhibition responses due to imprecise and unsteady positions of the coil. To reduce coil position and orientation variability, neuronavigated TMS (nTMS) could be used. We evaluated the accuracy of nTMS and a standardized function-guided procedure for maintaining TMS coil position both in unfatigued and fatigued knee extensors. Eighteen participants (10F/8M) volunteered in two identical and randomized sessions. Maximal and submaximal neuromuscular evaluations were performed with TMS three times before (PRE_1) and three times after (PRE_2) a 2 min resting session and one time immediately after (POST) a 2-min sustained maximal voluntary isometric contraction (MVIC). The located "hotspot" [the location that evoked the largest motor-evoked potential (MEP) responses in the *rectus femoris*] was maintained either with or without nTMS. MEP, silent period (SP) and the distance between the "hotspot" and the actual coil position were recorded. A time × contraction intensity × testing session × muscle interaction was not observed for MEP, SP, and distance. Bland-Altman plots presented adequate agreements for MEP and SP. Spatial accuracy of TMS coil position over the motor cortex did not influence corticospinal excitability and inhibition in unfatigued and fatigued knee extensors. The variability in MEP and SP responses may be due to spontaneous fluctuations in corticospinal excitability and inhibition, and it is not altered by the spatial stability of the stimulation point.

1. Introduction

During fatiguing contractions, the modulation of the motoneuronal excitatory drive to the muscle can be due to changes in supraspinal inputs and in the intrinsic properties of the motoneurons [1]. The state of the excitability or inhibition of the central nervous system can be detected with transcranial magnetic stimulation (TMS). Indeed, the evoked responses at the muscle level represent a measure of the amount of the central activation or inhibition [2]. Stimulation of the primary motor cortex using TMS results in a transient cortical excitation (motor-evoked potential, MEP) followed by a period of near-silence in the EMG signal (silent period, SP). However, MEPs and SPs present a high degree of variability [3] due to (e.g.) the position and size of the electrodes on the muscle [4], the level of muscle activation [5], and the type of coil used [6]. Importantly, the coil positioning (location, orientation, and tilt) may also affect the amplitude of the TMS-evoked responses, likely due to imprecise and unsteady positions of the coil during the testing sessions [6]. Accordingly, accurate and stable positioning of the coil is difficult to achieve, and various methods have been used in the literature.

To decrease the variability in TMS-evoked responses by controlling the coil positioning, studies have used neuronavigator systems. Navigated TMS (nTMS) has been developed for computer-assisted procedures and adapted to TMS. It employs anatomical data and an optically tracked frameless stereotaxic system [7] to integrate an individual's brain imaging data to better identify the motor cortex region of interest [8]. It gives then the possibility to continuously control for the optimal stimulation site ("hotspot") and maintain it with an accuracy of- 3 mm

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[9], limiting the localization errors and increasing the spatial accuracy (the reliability of the coil positioning during a series of TMS [10]) during and between TMS sessions [11].

In studying exercise-induced neuromuscular fatigability (a decline in the maximal force-generating capacity of a muscle [1]), the coil positioning during testing sessions are usually held using external landmarks on the skull [12–18]. However, the strength and accuracy of the evoked responses during the testing sessions may be modulated by a different coil positioning before and after fatiguing sessions. Accordingly, small changes in the coil positioning could result in significantly different corticospinal responses, highlighting the necessity of considering this variability to avoid suboptimal stimulation. This is an important consideration in the study of neuromuscular fatigability because coil mispositioning after fatiguing sessions could lead to maximizing the physical variation in stimulus delivery and minimizing the repetition of optimally targeted stimulation. This would lead to lesser stability of MEP responses and less consistent modulation of local cortical excitability and inhibition. The retention of the coil positioning over the "hotspot" with nTMS could therefore help to reduce the within- and between-session variability in the corticospinal responsiveness. However, whether a higher accuracy of nTMS is maintained with neuromuscular fatigability is unknown. This information is important if nTMS has to be used to quantify changes in corticospinal responsiveness after a fatiguing task.

We aimed to evaluate the accuracy of nTMS and a standardized function-guided procedure for the maintenance of coil positioning both in unfatigued and fatigued knee extensors. We tested the hypothesis that the use of nTMS resulted in greater accuracy and stability in the corticospinal responses in unfatigued and fatigued conditions.

2. Material and methods

2.1. Participants

An a priori sample size of nine individuals was determined using G-Power 3.1, assuming an anticipated effect size (f) of 0.25, which is considered medium [19], an alpha of 0.05, a beta of 0.2, a correlation among repetition measures of 0.5, and the nonsphericity correction ε of 1. To account for possible dropouts, eighteen healthy and physically active adults (10F/8M: 24 ± 3 years; 67 ± 14 kg; 170 ± 9 cm) volunteered for this study. Exclusion criteria were history of heart disease or hypertension, neurological disorders, lower-body injury in the previous 6 months, and contraindications to TMS [20]. Participants were informed about the experimental protocol and all associated risks prior to giving written informed consent. This study conformed to the Declaration of Helsinki, except for registration in a database, and was approved by the local Ethics Committee.

2.2. Experimental protocol

Participants visited the lab on three different occasions. They were asked to abstain from caffeine on the day of the experiment and avoid performing vigorous exercise at least 24 h prior to each laboratory session. During the first visit, participants completed questionnaires about risks associated with the use of TMS [21] and to assess leg dominance [22]. Thirteen and five participants had dominant right and left leg, respectively. During the familiarization, participants were instructed to extend their knee (in isometric conditions) "as hard as possible". Familiarization consisted in 5-to-10 non-successive contractions, allowing participants to feel comfortable with the procedures. They also familiarized with submaximal knee extensors contractions, TMS and femoral nerve stimulation. During the second and third visits, in a randomized and counterbalanced order, participants performed the same protocol, and an experienced investigator (CB) was either allowed or not to use nTMS to maintain the coil positioning on the "hotspot". To avoid the influence of different phases of the menstrual cycle on the neuromuscular assessment [23], the first day of menstruation was considered as day 1 of the cycle and women visited the lab on day 15 ± 3 of their menstrual cycle. The two test sessions were separated by 30 ± 5 days for all the participants, regardless of sex. Testing sessions were held at the same time of day for each participant to control for within-participant diurnal variation. Participants carried out a standardized warm-up consisting of three voluntary contractions at 10%, 30%, 50% and one at 70% of their maximal voluntary isometric contraction (MVIC) assessed during the familiarization. Each warm-up contraction was 5 s long, with 5 s of rest in between. The warm-up was followed by two 5-s isometric MVICs with 2 min of rest in between. Then participants performed six neuromuscular evaluations interspersed by at least 1 min of rest. The first three neuromuscular evaluations (PRE_1) were followed by a 2-min resting period and the coil was removed from the participants' head. At the end of the 2-min, the second three neuromuscular evaluations were performed (PRE_2). These procedures were conducted to control for the effect of coil repositioning on the corticospinal responsiveness, without the influence of fatigue. After PRE_2, participants sustained an MVIC for 2-min, immediately followed by the last neuromuscular evaluation (POST) (Fig. 1). Participants were strongly and verbally encouraged throughout the sessions.

2.2.1. Neuromuscular evaluation

The neuromuscular evaluation included four contractions: one 3–5 s MVIC, followed by 3–5 s contractions at 75% and 50% MVIC (to overcome the nonlinear behavior of MEP area for intensities below 50% MVIC [24]), and a 12–15 s contraction at 20% EMG. The 75% and 50% submaximal force targets were calculated from the corresponding MVIC, while the target for the 20% EMG was calculated from the root mean square of the EMGmax signal during the corresponding MVIC over a 200 ms time-window before TMS. Within each set, contractions were interspersed by 5 s. Participants were provided with visual feedback of the force produced and the target EMG signal on a computer screen using a custom-written MATLAB toolbox (The MathWorks, Inc., Natick, MA). During the MVIC (as well as the 75% and 50% MVIC), participants contracted to the required force level, and once it was achieved and plateaued, TMS was delivered. Participants were also asked to re-contract as quickly as possible to the pre-stimulus level of force after the TMS [25]. Once force plateaued again, femoral nerve stimulation was delivered. During the 20% EMG contraction, three TMS and one femoral nerve stimulation were delivered in a randomized order, interspersed by 3 s. All peak forces from the six MVIC trials were within 5% of each other during each of the two testing sessions.

2.2.2. Force recording

Force was measured by a force transducer (S2tech 560 QDT, Milan, Italy) previously calibrated and connected to a high-speed acquisition system (PowerLab 16/30; ML880, ADInstruments, Australia). Signal output was amplified (INT2-L, London Electronics Limited, Sandy, Bedfordshire, United Kingdom) and sampled at 2000 Hz. The participants were comfortably seated in a custom-made chair with a 90° knee flexion. The force transducer was connected to the chair's bar and located in front of the participants' ankle joint. The participant's dominant leg was placed in a U-shaped hold to ensure a foothold for the frontal part of the ankle 2 cm above the external malleolus. The participants were asked to push against a compression load cell.

2.2.3. EMG signal

Continuous EMG signals from the *rectus femoris*, *vastus lateralis*, and *biceps femoris* were recorded using three pairs of self-adhesive surface electrodes (Ambu Neuroline 715; Ambu A/S, Ballerup, Denmark) in a bipolar configuration with a 20-mm interelectrode distance and reference on the patella. The EMG electrodes were placed according to standardized procedures [26]. The EMG signals were acquired and amplified by Quad Bio Amp (ML135, ADInstruments, Australia) with a band pass filter (10–500 Hz). All signals were integrated and digitalized

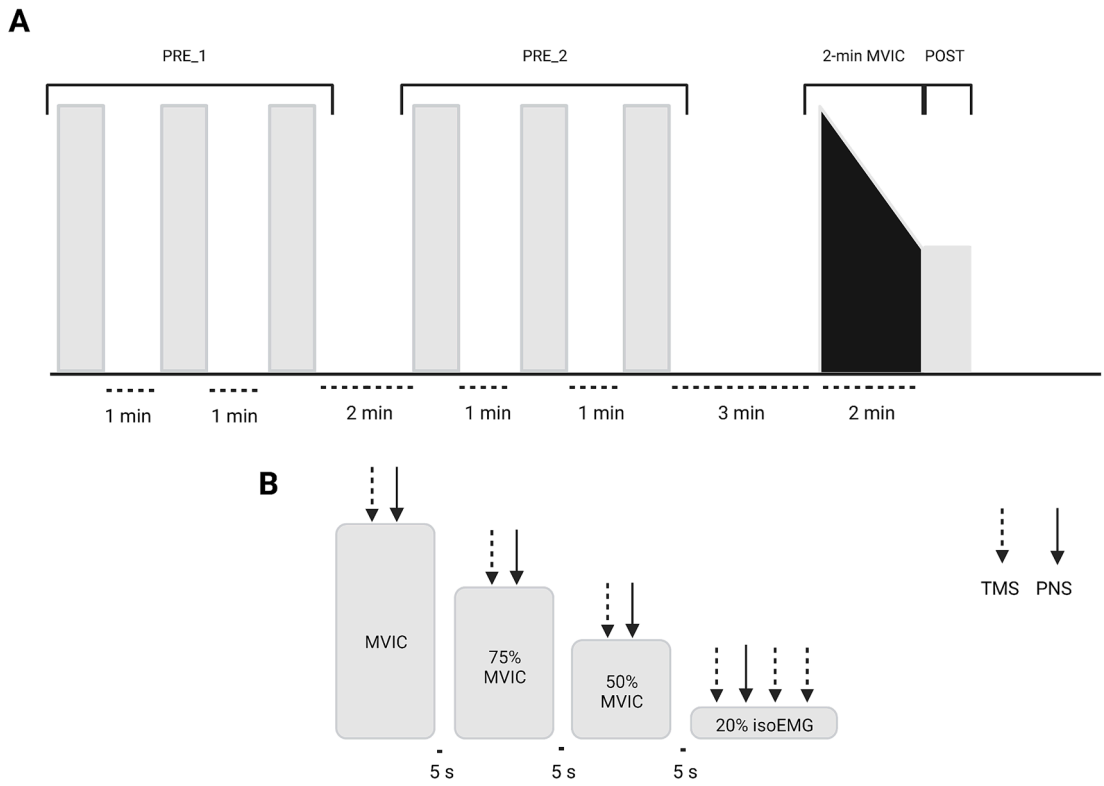

Fig. 1. (Panel A) Experimental protocol completed in two different laboratory sessions with or without navigated transcranial magnetic stimulation (nTMS). Participants performed neuromuscular evaluations three times before (PRE_1) a 2 min resting session. During the 2 min resting session, the TMS coil was removed and repositioned before performing the other three neuromuscular evaluations (PRE_2). After 3 min, participants performed a fatiguing task constituting of a 2-min sustained maximal isometric contraction (MVIC). Immediately at the end (i.e., participants were not allowed to relax), they performed one neuromuscular evaluation (POST). **(Panel B)** The neuromuscular evaluation required participants to perform a sustained MVIC. Guidelines at 75% and 50% of maximal force were instantaneously displayed on the computer screen so that the contraction was sustained at 75% MVC and then 50% MVC. Motor cortex and femoral nerve stimulations were delivered at each force level once the participants produced the appropriate amount of force. At the end of this set of contractions, a sustained submaximal contraction at 20% EMG was performed. During this contraction, three motor cortex and one femoral nerve stimulations were delivered in a randomized order, interspersed by 3 s. Within each set, contractions were interspersed by 5 s. Each set of contractions lasted approximately 15 s.

with PowerLab (16/30; ML880, ADInstruments, Australia) at a sampling rate of 2000 Hz.

2.2.4. *Transcranial magnetic stimulation*

Using a 110-mm double cone coil, single stimuli were delivered over the contralateral motor cortex with a magnetic stimulator (Magstim rapid^2, The Magstim Company Ltd, Whitland, UK), inducing a posterior-anterior current. To identify the vertex, the participants wore a tightly fitting white lycra swimming cap, and lines between preauricular points and from nasion to inion were drawn. Considering every centimeter, from the vertex to 2 cm posterior along the nasal-inion line and 1 cm laterally over the contralateral motor cortex, six points were drawn. For both testing sessions, an optically tracked frameless stereotaxic neuronavigation system (SofTaxic Navigator system, Electro Medical Systems, Bologna, Italy) was used to record coil position, orientation, and tilt for each stimulation. The coil positioning was recorded at each TMS trigger. During one session, the investigator controlled the coil positioning on the nTMS screen. During the other session, the investigator was not allowed to use the live feedback of the neuronavigation system, and the coil positioning was marked with a semi-permanent marker on the lycra-cap (to enable the investigator to reposition the coil correctly throughout). The nTMS can display and record all spatial information and brain target area thanks to an optical tracking system (Polaris Vicra, Northern Digital Inc., Canada) and two sets of spherical, *retro*-reflective markers placed on the coil handle and on the participant's forehead [7]. An individualized probabilistic head model was used to guide coil positioning. To create the head model, two sets of analogous cranial landmarks (nasion, left and right pre-auricular notches) were manually digitized from the participant's head using a stylus. The local coordinate system for the coil was also determined, specifying three points with a stylus: two described the transversal plane and one the origin. When the "hotspot" was identified, the same investigator had both spatial and numeric feedback provided through three configurable viewports. The feedback shown in the viewports was numeric (centimeters and degrees) and colored (red to green), and the investigator was able to check the matching accuracy of coil positioning with respect to the "hotspot" (determined as the point where the largest MEP amplitude in the *rectus femoris* was obtained during isometric muscle contraction at 20% MVIC at a 50% maximum stimulator output [12–14]). To calculate the distance between the point of stimulation and the "hotspot", the coordinates for each TMS trigger were recorded and then exported in XML format for offline analysis. The TMS intensity was determined by constructing a stimulus–response curve of four brief consecutive contractions each at 20%, 30%, 40%, 50%, 60%, 70%, and 80% of the maximum stimulator output in a randomized order. A plateau in the curve was not reached in three participants; therefore, a further intensity of 90% was used. The adequate intensity was defined as the one inducing maximal MEP amplitudes in the *rectus femoris* and the minimum responses in the *biceps femoris* [27] (Fig. 2). Mean stimulus intensities were 68 ± 12% and 68 ± 11% (p = 0.791) of maximum stimulator output for the TMS and nTMS testing sessions, respectively.

2.2.5. *Peripheral nerve stimulation*

Single electrical stimuli of 1 ms duration were delivered using a

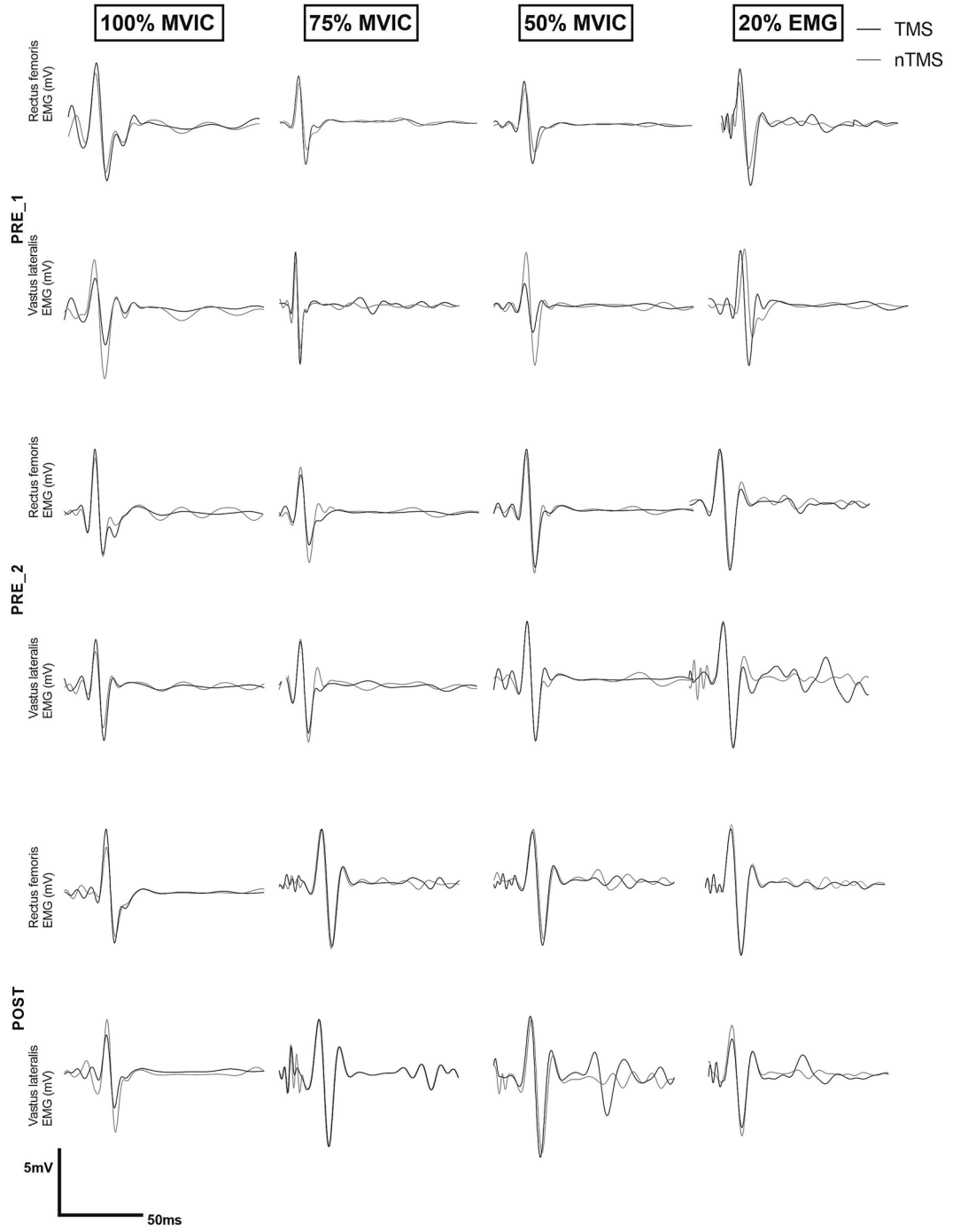

Fig. 2. Single-participant data showing raw electromyographic (EMG) responses evoked by transcranial magnetic stimulation in the *rectus femoris* and *vastus lateralis* during neuronavigator (nTMS) and non-neuronavigator (TMS) sessions before (PRE_1) and after (PRE_2) a 2 min resting session, as well as immediately after (POST) a 2-min sustained maximal voluntary isometric contraction (MVIC).

constant-current stimulator (Digitimer DS7AH, Welwyn Garden City, United Kingdom) to the femoral nerve. Stimuli to the femoral nerve were delivered via a surface 3.2 cm round cathode (StimTrode, Axeelgaard Manufacturing, Fallbrook, CA) securely taped into the femoral triangle and a 3.2 cm round anode (StimTrode, Axeelgaard Manufacturing, Fallbrook, CA) placed between the greater trochanter and the iliac crest. Single stimuli were delivered incrementally in a relaxed muscle state until the compound muscle action potential's amplitude (M−wave) of *rectus femoris* (as well as twitch amplitudes) plateaued. A stimulus intensity of 120% of the intensity to elicit maximal M−wave amplitude (Mmax) and maximal twitch responses was used throughout the rest of the experiment. Stimulus intensity was determined at the start of each session. Mean stimulus intensities were 220 ± 69 mA and 195 ± 38 mA for the TMS and nTMS testing sessions, respectively ($p = 0.352$).

2.3. Data analysis

Force values were calculated as the difference between the baseline and the average of a 500 ms time-window before the TMS [14]. Area values for MEP and Mmax were measured between cursors marking the initial deflection from the baseline to the second crossing of the horizontal axis [28]. We only reported MEP area to control for possible

confounding effects of amplitude and/or duration of the MEP responses. To account for any changes in the sarcolemmal excitability, MEP was normalized to the compound muscle action potential (Mmax) values (MEP/Mmax) recorded during the same contraction. The SP duration was measured by visually inspecting the interval from the stimulus to the return of continuous voluntary EMG [29]. The distance between the "hotspot" and the stimulation point was calculated using the 3D Euclidean distance formula of two points, using the coordinates recorded during the session [9]. This distance can affect the excitability results (the higher the distance, the lower the MEP amplitude [30]) more than the coil tilt or orientation [31].

2.4. Statistical analysis

Normality of the data was confirmed by Shapiro-Wilk W test. A two-way repeated measures ANOVA [time (PRE_1, PRE_2 and POST) × testing session (TMS and nTMS)] was employed to test possible differences in the MVIC force. To assess the within-participant stability of corticospinal responses, the coefficient of variation (CV = 100 × SD/mean) values of MEP/Mmax and SP were computed for PRE_1 and PRE_2 for each contraction intensity and muscle. To interpret the CV values, an analytical goal of ≤ 15% was used [32]. To assess relative reliability, and the corresponding 95% confidence interval (95% CI), two-way random effects, absolute agreement intraclass correlation coefficients ($ICC_{2,1}$) were also calculated. As a general rule, we considered values from 0.7 to 0.8, from 0.8 to 0.9, and > 0.9 questionable, good, and high, respectively [33]. Then four-way repeated measures ANOVA [time (PRE_1, PRE_2 and POST) × contraction intensity (100%, 75% and 50% MVIC) × testing session (nTMS and TMS) × muscle (*rectus femoris* and *vastus lateralis*)] was employed to test possible differences in the variables of interest (CV, MEP/Mmax, SP and distance). The assumption of sphericity was checked using Mauchly's test and it was not violated for all the variables of interest ($p > 0.05$). Significant main and interaction effects were explored using Bonferroni-corrected tests. For PRE_1, PRE_2 and POST, and for each contraction intensity, Bland-Altman plots were drawn to establish the bias of MEP/Mmax and SP values determined from the TMS session and the nTMS session [34]. To do so, we plotted the difference between the two testing sessions against their mean [34] and considered the values from the nTMS sessions as the criterion variable. Examination of the direction and magnitude of the scatterplot around the zero line provides an approximate indication of the systematic bias and random error, respectively [35]. Confidence intervals defining the limits of agreement were established as ± 1.96 SD from the mean difference as an index of random error. An additional analysis was conducted to explore whether the degree of systematic error was uniform over the two sessions studied. To do so, a regression analysis was applied to model the relationship between the size of the mean measured values and individual participant differences between the sessions [36].

Statistical analyses were conducted using SPSS (version 28.0.0, IBM Corp., Somers, New York, NY). The level of significance was set at $\alpha < 0.05$.

3. Results

3.1. Force

MVIC force data showed a time effect ($df = 1.042$, $F = 216.758$, $p < 0.001$), but not a testing session effect ($df = 1$, $F = 0.684$, $p = 0.414$) or an interaction ($df = 1.042$, $F = 4061.081$, $p = 0.587$). MVIC force data at PRE_2 (469 ± 154 N) was similar to PRE_1 (461 ± 149 N) ($p = 0.157$); however, both were ~ 75% greater than POST ($p < 0.001$) (Fig. 3). The ICC value for force was 0.912 (95% CI 0.830–0.963).

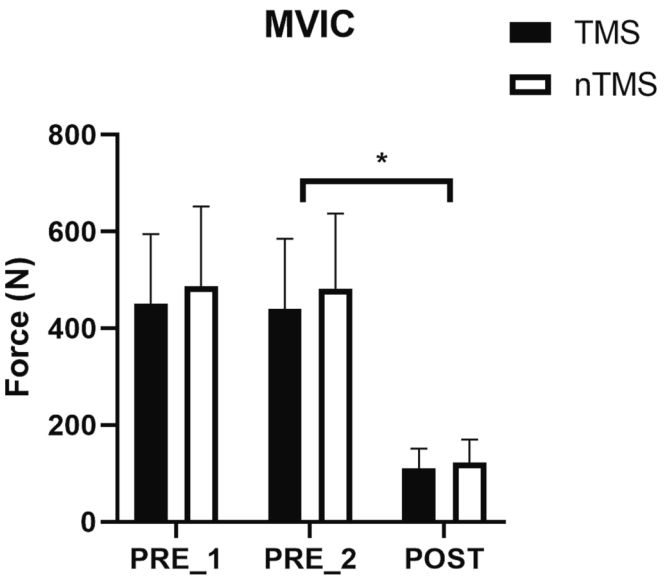

Fig. 3. Participants' maximal voluntary isometric contraction forces before (PRE_1) and after (PRE_2) a 2 min resting session, as well as immediately after (POST) a 2-min sustained maximal voluntary isometric contraction. The values represent group means and standard deviations during neuronavigator (nTMS) and non-neuronavigator (TMS) sessions. * Time effect from PRE_2 ($p < 0.001$).

3.2. Corticospinal excitability, inhibition, and distance

CV of MEP/Mmax ($df = 3$, $F = 2.233$, $p = 0.085$) and SP ($df = 3$, $F = 0.183$, $p = 0.908$) did not show a time × muscle × contraction intensity × testing session interaction between PRE_1 and PRE_2. Furthermore, MEP/Mmax ($df = 4.244$, $F = 1.079$, $p = 0.368$) and SP ($df = 3.773$, $F = 0.036$, $p = 0.997$) did not show a time × muscle × contraction intensity × testing session interaction. Distance did not show a time × contraction intensity × testing session interaction ($df = 4.869$, $F = 0.297$, $p = 0.911$) (Supplementary Table S1). Considering the four contractions intensity, the three time points (PRE_1, PRE_2 and POST) and the two different sessions, the ICC values for MEP/Mmax, SP, and Distance were 0.951 (95% CI 0.924–0.971), 0.953 (95% CI 0.927–0.972), and 0.942 (95% CI 0.895–0.974), respectively.

3.3. Bias of MEP, SP, and distance

Individual differences were plotted against individual means for the MEP/Mmax and SP from the session with and without nTMS at PRE_1 (Supplementary Figure S1), PRE_2 (Supplementary Figure S2), and POST (Supplementary Figure S3). Overall, the Bland-Altman plots display an adequate agreement at all time points for MEP/Mmax and SP at all contraction intensities from the session with and without nTMS. The slopes of the regression lines from these analyses were not significantly different from zero (horizontal to *x*-axis) ($p \geq 0.068$), showing uniformity of systematic error.

4. Discussion

We compared the accuracy between navigated TMS and a standardized function-guided procedure for the maintenance of coil positioning during testing sessions for the assessment of corticospinal responsiveness in unfatigued and fatigued knee extensors. Results suggest that the assessment of corticospinal excitability and inhibition using nTMS is as accurate as a standardized function-guided procedure. Therefore, we reject our hypothesis that the use of nTMS would have resulted in greater accuracy and stability in the corticospinal responses in unfatigued and fatigued conditions.

Using a standardized function-guided procedure method, the

retention of the coil positioning during testing sessions is accurate only if the distance between the center of the coil and the primary motor cortex is minimal [37], and if the coil is correctly oriented [38]. For example, during the MVICs we observed a mean distance between the nTMS and the standardized function-guided procedure of 0.51 ± 5.19 mm, 1.03 ± 4.37 mm, and 0.40 ± 5.50 mm for PRE_1, PRE_2, and POST, respectively. Accordingly, we observed a narrow spatial dispersion of the points and a narrow variation in the distance between nTMS and a standardized function-guided procedure. Therefore, TMS coil positioning with the standardized function-guided procedure is suitable both in unfatigued and fatigued conditions.

Although not unanimously agreed upon [9,10,39], our results suggest that spatial accuracy in the millimeter range seems to not influence the TMS-evoked responses in unfatigued conditions, as previously observed [6,40]. Indeed, corticospinal responsiveness was similar between the sessions performed with or without the use of nTMS and across maximal and submaximal contraction intensities. Furthermore, the relative stability of these responses (assessed with the coefficient of variation) was similar. The TMS-evoked responses can be highly variable because of independent fluctuations in the excitability of motor cortex neurons, spinal interneurons and motoneurons [3]. Therefore, controlling the TMS coil positioning over the intended area with nTMS could have helped to reduce this variability. However, this was not the case here since the level of spatial accuracy did not influence the level of spontaneous and independent fluctuations in corticospinal responses. Therefore, our results emphasize that stable positioning of the coil using a function-guided procedure is accurate enough to detect TMS-evoked responses in unfatigued conditions.

The real-time visualization and feedback of the TMS coil positioning provided by nTMS during testing sessions should lead to superior targeting and stabilization of stimulus delivery relative to a standardized function-guided procedure, particularly in fatigued conditions. However, we showed that similar precision in the TMS coil placement can be achieved either with or without nTMS during testing sessions. Therefore, a standardized function-guided procedure seems to be accurate enough to not affect the mean MEP and SP responses at the group level in fatigued conditions.

In conclusion, function-guided procedure seems to be accurate enough to not affect the corticospinal responsiveness at the group level in unfatigued and fatigued conditions. This procedure is easily applicable and could be applied in the study of excitatory (motor-evoked potential) and inhibitory (silent period) responses elicited by TMS in fatigued conditions. The main advantage is that it easily allows investigators to align the center of the TMS coil with the target site. In this way, a constant coil position over time can be assured. However, this procedure can be recommended if an adequately trained investigator can reliably identify the target region, as well as position and hold the TMS coil.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neulet.2023.137351.

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