Voluntary activation of knee extensor muscles with transcranial magnetic stimulation

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25 **NEW & NOTEWORTHY**

26 We discovered that the contemporary procedure for assessing voluntary activation of the knee
27 extensor muscles with transcranial magnetic stimulation (TMS) is invalid. TMS activates too
28 few agonist quadriceps motoneurons and too many antagonist hamstrings motoneurons to
29 estimate the resting twitch accurately. A modified procedure, in which TMS-evoked
30 superimposed twitches are considered together with the resting twitch from femoral nerve
31 stimulation, is valid, but only in select individuals who meet rigorous eligibility criteria.
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ABSTRACT

We examined if transcranial magnetic stimulation (TMS) is a valid tool for assessment of voluntary activation of the knee extensors in healthy individuals. Maximal M-waves (M_{max}) of vastus lateralis (VL) were evoked with electrical stimulation of femoral nerve (FNS); M_{max} of medial hamstrings (HS) was evoked with electrical stimulation of sciatic nerve branches; motor evoked potentials (MEPs) of VL and HS were evoked with TMS; superimposed twitches (SIT) of knee extensors were evoked with FNS and TMS. In Study 1, TMS intensity (69% output (SD 5)) was optimized for MEP sizes, but guidelines for test validity could not be met. Agonist VL MEPs were too small (51.4% M_{max} (SD 11.9); guideline ≥70% M_{max}) and antagonist HS MEPs were too big (16.5% M_{max} (SD 10.3); guideline <10% M_{max}). Consequently, the TMS estimated resting twitch (99.1 N (SD 37.2)) and FNS resting twitch (142.4 N (SD 41.8)) were different. In Study 2, SITs at 90% maximal voluntary contraction (MVC) were similar between TMS (16.1 N (SD 10.3)) and FNS (20.9 N (SD 16.7)), when TMS intensity was optimized for this purpose, suggesting a procedure that combines TMS SITs with FNS resting twitches could be valid. In Study 3, which tested the TMS intensity (56% output (SD 18)) that evoked the largest SIT at 90% MVC, voluntary activation from TMS (87.3% (SD 7.1)) and FNS (84.5% (SD 7.6)) were different. In sum, the contemporary procedure for TMS-based voluntary activation of the knee extensors is invalid. A modified procedure improves validity, but only in individuals who meet rigorous inclusion criteria for SITs and MEPs.

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INTRODUCTION

Voluntary activation is the nervous system's ability to drive a muscle to create its maximal force (13). Activation is often assessed with the interpolated twitch method (13, 20, 22, 27, 32, 35). Historically, the interpolated twitch method has involved the application of an electrical stimulus to a peripheral nerve during a maximal voluntary contraction (MVC) and then again after the MVC with the muscle at rest (13, 20). Activation is then computed by comparing the size of the superimposed twitch (SIT) during the MVC to the size of the potentiated resting twitch with the muscle at rest: voluntary activation (%) = (1 – SIT/potentiated resting twitch) x 100. If the superimposed stimulus evokes force during the MVC, then the individual was not voluntarily recruiting all of their motoneurons, or their motoneurons were discharging at subtetanic rates, and activation is less than 100%. Marked reductions in activation are characteristic of acute muscle fatigue (13) and are found in neurologic conditions such as stroke (7, 17, 21), multiple sclerosis (24, 39), and prior polio (1, 2, 6). Thus, the measure has been of growing interest to biological and rehabilitation scientists in the past 40 years (22).

One limitation of the interpolated twitch with electrical stimulation of the peripheral nerve is that it cannot reveal the site of the motor pathway responsible for a change in activation. In 2003, Todd and colleagues addressed this limitation by using transcranial magnetic stimulation (TMS) over the motor cortex to evoke SITs of the elbow flexor muscles (36). This TMS technique is reliable and valid for the elbow flexors (36, 37) and can test if voluntary output from the cortex is suboptimal during MVCs – that is, if motoneuron recruitment and/or motoneuron firing rates are below baseline or control levels as a consequence of suboptimal descending drive. The between-sessions reliability for the estimated resting twitch from this technique is $r \geq 0.98$ (37). In recent years, researchers have adopted this TMS procedure to test voluntary activation of the *knee extensor* muscles (4, 5, 8,

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12, 14-16, 19, 23, 25, 26, 28-31, 33, 34, 40, 41). However, the validity of the TMS procedure to test voluntary activation of the knee extensors has not been properly examined.

For TMS to provide a valid measure of voluntary activation of the knee extensors, the TMS pulse must evoke a sufficient SIT of the knee extensors. This will occur if the position of the magnetic coil on top of the head and the level of stimulator output (hereafter termed "TMS intensity") cause sufficient activation of the motor area of the agonist quadriceps during contractions of the knee extensors, while simultaneously causing minimal activation of the motor area of the antagonist hamstrings. If the TMS pulse activates too little of the agonists and/or too much of the antagonists, the size of the SIT will be reduced, and the computed voluntary activation will be overestimated given that the resting twitch is the same in the conditions compared. Moreover, because TMS voluntary activation involves estimation of the resting twitch from linear regression between the voluntary forces and SITs across a range of contraction strengths, if TMS evokes relatively small quadriceps motor evoked potentials (MEPs), the slope of the regression may be flattened and lead to underestimation of the size of the resting twitch and consequently a lower than expected level of voluntary activation. By contrast, relatively large hamstrings MEPs are more likely to lead to a steeper regression, larger estimated resting twitch, and overestimation of voluntary activation.

To gauge the degree to which the TMS pulse activates the motor areas and motoneuron pools of the agonist quadriceps and antagonist hamstrings, sizes of MEPs from agonist and antagonist muscles can be expressed as a percentage of the maximal compound muscle action potential (M_{max}) from the same muscles. Current recommendations for TMS-based voluntary activation are that the amplitude of the agonist MEP should be ≥70% of M_{max} during a 50% MVC contraction of the agonist and that the amplitude of the antagonist MEP should be ≤10% of M_{max} during an MVC of the agonist (22), although more liberal criteria have also been proposed (agonist MEP >50% M_{max}; antagonist MEP <20% M_{max}) (35). Thus,

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in setup procedures for an experiment, the investigator should identify the TMS intensity that is optimal for evoking large MEPs in the quadriceps and small MEPs in the hamstrings, which should correspond to the largest-possible SITs of the knee extensors. However, the application of the contemporary TMS procedure to the knee extensors is likely problematic. First, no matter what TMS intensity is used, the TMS pulse might simply be incapable of activating the quadriceps motor area and motoneuron pool at a sufficient level. For example, Dekerle et al. (9) recently reported that VL MEPs are less than 50% M_{max} when tested during knee extensor contraction strengths of 50%, 75%, and 100% MVC. Second, the degree to which the TMS pulse might inadvertently activate the hamstrings motor area and motoneuron pool is unknown. This is because the acquisition of the hamstrings M_{max} has rarely been attempted. In 2009, Sidhu et al. (28) obtained M_{max} from biceps femoris via the application of high-intensity electrical stimulation (~650 mA) over the sciatic nerve. However, because of stimulation discomfort, M_{max} was acquired in only 3 participants (28), and no subsequent studies have used this stimulation technique. Lack of acquisition of hamstrings M_{max} has been a key issue in previous studies on TMS-based voluntary activation of the knee extensors, but it is not the only one. In Table 1, we summarize the various methods and criteria that have been used in such studies. Issues in all or some of the studies summarized include: (a) hamstrings M_{max} not acquired; (b) hamstrings MEPs not acquired; (c) hamstrings MEPs acquired, but the degree to which they represent an acceptable level of activation has involved comparison to a measure other than hamstrings M_{max} (e.g., expressing the hamstrings MEP relative to the quadriceps MEP or quadriceps M_{max}); (d) SITs not considered when determining TMS intensity; and (e) contraction intensities used when determining TMS intensities are too low (e.g., 10 or 20% MVC).

To summarize, the validity of TMS-based voluntary activation of the knee extensors remains unknown because a reliable technique for evoking hamstrings M_{max} has not been

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established. Consequently, it has not been possible to determine the degree to which the TMS pulse might inadvertently activate the hamstrings motor area and motoneuron pool, and subsequently impact SITs of the knee extensors. Moreover, the degree to which the TMS activates the agonist quadriceps appears problematic (9) and warrants further investigation. Therefore, the overarching purpose of the current investigation was to determine if TMS is a valid tool for the assessment of voluntary activation of the knee extensors.

In Study 1, our aims were to (a) develop a reliable method for evoking hamstrings M_{max} and (b) describe evoked responses (hamstrings MEP/M_{max}, quadriceps MEP/M_{max}, SITs) across a range of contraction strengths using a single "optimal" TMS intensity based on current guidelines (22); and (c) determine if the estimated resting twitch from the TMS procedure is similar to the actual resting twitch from femoral nerve stimulation (FNS). We discovered the single "optimal" TMS intensity activated too few quadriceps motoneurons and too many hamstrings motoneurons. Consequently, the estimated resting twitch from TMS was different from the actual resting twitch from FNS. Therefore, in Study 2, we abandoned the notion that an accurate estimate of the resting twitch for the knee extensors can be derived from the contemporary TMS procedure. Instead, our aim in Study 2 was to determine if TMS intensities other than the "optimal" one might evoke SITs at high voluntary forces comparable to SITs evoked by FNS. If the TMS- and FNS-evoked SITs are comparable, then future studies could employ a modified TMS procedure that combines TMS-evoked SITs with the resting twitch from FNS to measure voluntary activation. After testing multiple TMS intensities, we discovered that TMS- and FNS-evoked SITs could be made similar (i.e., TMS/FNS SIT ratio ≥0.80) in ~60% of participants if TMS intensity is optimized for SITs at 90% MVC. Therefore, in Study 3, we aimed to determine if the TMS/FNS SIT ratio, along with quadriceps and hamstrings MEPs, could be used to identify individuals in whom the modified TMS procedure provides a valid measure of voluntary activation.

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MATERIALS AND METHODS

Ethical approval

Studies 1 and 2 were approved by the Human Research Ethics Committee at the University of New South Wales (HC14316). Study 3 was approved by the Human Research Ethics Committee at Edith Cowan University (2019-00256-Taylor). The studies were conducted in compliance with the *Declaration of Helsinki* (2008), except for registration in a database. All individuals provided their written informed consent to participate.

Participants

Participants for Studies 1 and 2 were recruited via paper advertisements posted at the University of New South Wales, and participants in Study 3 were recruited via paper and electronic advertisements posted at Edith Cowan University (Joondalup campus). Individuals were excluded from participation if they were not between the ages of 18–50, were pregnant, or were taking medications; had a history of epilepsy, frequent headaches, or fainting; had undergone a surgical procedure of the brain; had a neurostimulator in the brain or other metal in the brain or skull; had a history of brain-related conditions (e.g., stroke, head trauma that caused loss of consciousness); had hearing problems or ringing in the ears; or had cochlear implants or cardiac pacemakers in their bodies.

Studies 1 and 2 were undertaken by the same participants. Thirteen participants (mean age: 24.8 years (SD 6.2); 8 males, 5 females) attended the laboratory for Study 1. M-waves of the medial hamstrings (HS) could not be obtained in one male participant, and no further tests were completed with him. The final sample for Study 1 was 12. One female participant who completed Study 1 elected not to participate in Study 2. The final sample for Study 2 was 11.

For Study 3, 21 individuals (mean age: 26.1 years (SD 5.3); 17 males, 4 females) attended the laboratory. Five individuals withdrew during test procedures, usually due to

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177 stimulation discomfort. HS M-waves could not be obtained in two male participants and no
178 further tests were completed in them. The remaining 14 participants underwent the
179 procedures in which the validity of TMS was assessed. Of these 14, 8 were eligible to
180 complete the entire experimental protocol. See Results for more details.

181 Participants in Studies 1-3 verbally reported a range of experiences with physical
182 exercise. Some had limited or no previous exercise experience, whereas others partook in
183 resistance training, cycling, and/or jogging. Of the 13 participants in Studies 1 and 2, 11 were
184 right leg dominant (i.e., leg used to kick a ball). All 21 participants in Study 3 were right leg
185 dominant.

186 **Experimental setup**

187 Participants were seated upright in a custom chair with hips and knees at 90°
188 (Fig. 1*A*). The chair consisted of a backrest, a seat, and a padded "knee bar." Between the
189 most distal portion of the seat and the most proximal portion of the knee bar was a gap that
190 permitted access to the posterior thigh for stimulation of sciatic nerve branches. Also, a
191 section of the pad on the knee bar was removed to allow for recording electrodes to be placed
192 on the distal HS without the electrodes being compressed down onto the knee bar. The right
193 ankle was strapped into a cuff which was connected to a force transducer (Studies 1 and 2:
194 linear to 2 kN XTran; Study 3: UU80-250N; Applied Measurement, Mitcham, Australia) via
195 a rope. A computer monitor was positioned in front of the participants to provide visual
196 feedback of voluntary force.

197 **Surface EMG**

198 Muscle activity was recorded from pairs of surface electrodes (~40mm inter-electrode
199 distance, Ag-AgCl, Conmed Cleartrace, Conmed Corporation, Utica, United States) over the
200 right vastus lateralis (VL), vastus medialis (VM), and HS. Palpation during weak muscle

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contractions guided electrode placement. Electrodes for VL were placed on the distal third of the muscle belly. Electrodes for VM were placed over the muscle belly and in the oblique direction of the fibers. Electrodes for HS were placed over the distal portion of the medial hamstrings and likely recorded from both semitendinosus and semimembranosus. The distal electrode placement for HS was necessary because stimulation artifact was an issue when the HS recording electrodes were positioned more proximal (i.e., closer to the site of stimulation). Recording electrodes for VL, VM, and HS were grounded to an electrode (3M Universal Electrosurgical Pad) placed over the patella. All EMG signals were amplified and filtered (×100; 16-1,000 Hz; CED 1902 amplifier, Cambridge Electronic Design, Cambridge, United Kingdom). The sampling rate was 2,000 Hz. Analog signals were digitized and stored on a laboratory computer using an interface (CED 1401 and Spike software, Cambridge Electronic Design).

Sciatic nerve stimulation (SNS)

A constant current stimulator (DS7AH, Digitimer, Welwyn Garden City, UK) was used to deliver electrical stimuli (500 μs pulse duration) to sciatic nerve branches to evoke HS M-waves. The cathode was a 12 mm diameter probe held in the hand of the investigator. The optimal position for the cathode differed between participants, but generally was on the lateral or mid-lateral aspect of the posterior thigh, about halfway along the length of the femur (Fig. 1*B*). The hamstring muscles and surrounding adipose tissue sagged with participants seated in the chair, so the probe was pushed firmly upward by the investigator to ensure adequate contact with the nerve. The anode was a 70 mm by 40 mm electrode (3M Universal Electrosurgical Pad) placed just distal to the gluteal fold. In Studies 1, 2, and 3, the mean stimulation intensities required to evoke HS M_{max} were 216.7 mA (SD 53.3; range: 160 – 340), 205.0 mA (SD 32.6; range: 160 – 280), and 180.6 mA (SD 46; range: 120 – 240), respectively.

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226 **Femoral nerve stimulation (FNS)**

227 A constant current stimulator (DS7AH) was used to deliver electrical stimuli (500 μs
228 pulse duration) to the femoral nerve to evoke VL and VM M-waves and SITs of the knee
229 extensors. The cathode was a custom circular probe (20 mm diameter) placed over the right
230 femoral nerve at the inguinal triangle. It was secured into position via an adjustable strap
231 around the thigh. The anode was a 70 mm by 40 mm electrode (3M Universal Electrosurgical
232 Pad) over the right iliac crest. Stimulation intensity was 1.2x the minimal level necessary to
233 evoke M_{max} in VL and VM. In Studies 1, 2, and 3, the mean stimulation intensities used
234 throughout testing to evoke VL and VM M_{max} were 129.0 mA (SD 49.1; range: 60 – 192),
235 156.7 mA (SD 47.9; range: 96 – 264), and 106.5 mA (SD 37.6; range: 48 – 132).

236 **Transcranial magnetic stimulation (TMS)**

237 A magnetic stimulator (Bistim^2, Magstim, Whitland, UK; stimulators discharged
238 simultaneously) and double-cone coil (11 cm outside diameter, Magstim) were used to evoke
239 MEPs and SITs of the right leg. The coil was held by the investigator over the left motor
240 cortex in an orientation that induced posterior-anterior current in the brain. The position of
241 the coil was that which evoked the largest VL MEPs with the muscle at rest or during a weak
242 voluntary contraction (5-10% MVC). This position was marked on a swim cap worn by the
243 participant and permitted consistent placement of the coil throughout the protocol.

244 In Study 1, we aimed to determine the ability of TMS to evoke MEPs and SITs of the
245 knee extensors using a single "optimal" TMS intensity based on current guidelines (i.e.,
246 agonist MEPs ≥70% M_{max} at 50% MVC; antagonist MEPs ≤10% M_{max} at 100% MVC) (22).
247 First, amplitudes of VL MEPs were assessed during brief knee extensor contractions at 50%
248 MVC. However, VL MEPs ≥70% M_{max} were generally not obtained (see Results). Thus, the
249 TMS intensity that evoked the largest VL MEP (usually 50-57% M_{max}) was identified and
250 then used to examine the sizes of HS MEPs at 100% MVC. HS MEPs were often >10% M_{max}

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(see Results), and lower TMS intensities to achieve smaller HS MEPs caused smaller VL MEPs. Thus, in Study 1, the "optimal" TMS intensity (69% (SD 5)) was typically that which evoked the largest VL MEPs at 50% MVC. However, the effect of TMS intensity on HS MEPs was always considered, particularly when two TMS intensities evoked VL MEPs of similar amplitude but HS MEPs of different amplitudes.

In Study 2, we aimed to determine if TMS intensities other than the "optimal" one might evoke SITs at high voluntary forces comparable to SITs evoked by FNS. We examined five TMS intensities. First, we established active motor threshold (AMT) for VL MEPs. AMT was the lowest TMS intensity that evoked ≥3 (out of 6) VL MEPs that were ≥0.5 mV in peak-to-peak amplitude during 10% MVC contraction. The 6 TMS pulses were separated by 10 seconds. Second, we established the "optimal" TMS intensity using the procedure from Study 1. Third, we calculated five different TMS intensities (TMS 1, 2, 3, 4, 5) to use in subsequent testing. "TMS 1" was AMT TMS intensity + 25% of the difference between the "optimal" TMS intensity and the AMT intensity. For example, if the "optimal" TMS intensity for a participant was 70% of stimulator output, and the AMT intensity for the same participant was 36% of stimulator output (difference of 34% output), then "TMS 1" was set at 45% of stimulator output (i.e., 36% + (34% x 0.25)). "TMS 2" was AMT intensity + 50% of the difference; "TMS 3" was AMT intensity + 70% of the difference; "TMS 4" was AMT intensity + 85% of the difference; and "TMS 5" was the "optimal" intensity. The average TMS intensities for "TMS 1," "TMS 2," "TMS 3," "TMS 4," and "TMS 5" were 43% (SD 6), 52% (SD 6), 58% (SD 7), 63% (SD 7), and 68% (SD 8), respectively.

In Study 3, we aimed to determine if SITs and MEPs could be used to identify individuals in whom TMS will provide a valid measure of voluntary activation of the knee extensors. Thus, evoked responses from TMS were part of the eligibility criteria for Study 3. Our primary criterion for eligibility was the size of the SIT from TMS at 90% MVC.

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More specifically, we examined the TMS SIT / FNS SIT ratio. For example, if the TMS SIT at 90% MVC was 15 N and the FNS SIT at 90% MVC was 17 N, the SIT ratio was 0.88. Results from Study 2 indicated that a SIT ratio ≥0.80 generally corresponded with VL and VM MEPs >40% M_{max} and HS MEPs <15% HS M_{max} at 90% MVC. Therefore, in Study 3, participants were deemed ineligible to undergo testing for voluntary activation if no TMS intensity led to a SIT ratio ≥0.80 at 90% MVC. For the 8 participants who were deemed eligible to undergo the full experimental protocol for testing of voluntary activation, the TMS intensity (56% (SD 18)) that produced the highest SIT ratios (termed the "high SIT ratio" TMS intensity) ranged from 35% of stimulator output for one participant to 85% of stimulator output for another participant. Note, for Study 3, we made the criteria for HS MEPs (≤15% M_{max} at 90% MVC) more liberal than the guideline used in Studies 1 and 2, which was ≤10% M_{max} at 100% MVC. The criteria were made more liberal in Study 3 because results from Study 2 indicated an adequate SIT of the knee extensors could be obtained if HS MEPs were between 10 and 15% HS M_{max}.

Experimental protocol

Three studies were conducted (Fig. 2). Study 1 consisted of one testing session. The setup procedures consisted of five steps. First, we assessed HS M_{max}. This procedure began by moving the stimulating probe to various positions at the back of the leg until the best position for evoking HS M-waves was identified. The best position was loosely defined as the point on the back of the leg, when stimulated at a given intensity, evoked the largest HS M-wave that was not obscured by the stimulus artefact. Stimulation intensity was then increased in increments of 10-30 mA until the HS M-wave no longer increased in amplitude (i.e., M_{max}). Time between each stimulus was about 10 to 20 seconds. Second, HS M-wave and H-reflex recruitment curves were established. Three stimuli were delivered to the sciatic nerve branches with 13 different SNS intensities. Intensities ranged from 10% to 130% of the

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minimum intensity necessary to obtain M_{max} from the first step. The procedure started with 3 stimuli delivered at the lowest intensity (10%) and then progressed in 10% increments until 3 stimuli were delivered at each intensity. Stimuli were delivered every 10 seconds. During the recruitment curve procedure, the probe was not removed from contact with the participant. It was held by the investigator in the same position until the test was completed. Third, we assessed VL and VM M_{max}. This procedure started with a stimulation intensity of 20 mA and the intensity was increased in increments of 20-30 mA until both VL and VM M-waves no longer increased in amplitude. Time between each stimulus was about 10 to 20 seconds. Fourth, participants performed maximal voluntary contractions (MVC) of the right knee extensors. Verbal encouragement and visual feedback were provided. Participants performed 3 MVCs with a 2-min rest between each. The highest of the 3 MVCs was used to calculate the submaximal force targets used in subsequent testing. Fifth, the "optimal" TMS intensity was identified using procedures described earlier (see Transcranial Magnetic Stimulation).

After Study 1 setup procedures were completed, participants performed 5 sets of voluntary contractions of the knee extensors. The first part of a set consisted of TMS-evoked responses during contractions at 100% MVC, 70% MVC, and 25% MVC, with the potentiated resting twitch from FNS occurring immediately after the contraction at 100% MVC. The second part of the set, which occurred after a 2-min rest, consisted of TMS-evoked responses during contractions at 85% and 50% MVC. The next set then commenced after another 2-min rest. The magnet was discharged by the investigator when the participant reached the target forces. Target forces were achieved within 2 seconds of contraction onset.

Study 2 involved one testing session and many of the same measurements and procedures as Study 1. After setup procedures to establish HS M_{max}, VL and VM M_{max},

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MVCs of the knee extensors, and TMS intensities, participants completed 18 sets of knee extensor contractions. Each set consisted of contractions at 90% and 50% MVC. The sets differed in regard to stimulation condition. Three sets were completed with TMS 1, TMS 2, TMS 3, TMS 4, TMS 5, and FNS. The order of the stimulation intensities was randomized. A 2-min rest was given between sets.

Study 3 consisted of one testing session and included many of the same measurements and procedures as Studies 1 and 2. After setup procedures for HS M_{max}, VL and VM M_{max}, MVCs of the knee extensors, and TMS eligibility procedures and identification of the "high SIT ratio" TMS intensity (see Transcranial Magnetic Stimulation), eligible participants completed 10 sets of knee extensor contractions. Five sets involved use of the "high SIT ratio" TMS intensity to evoke MEPs and SITs. The other five sets involved use of FNS to evoke SITs. Each set started with an MVC and was followed by contractions at 75% and 25% MVC. The contractions were separated by ~5 seconds. Following a 2-min rest, the participant completed contractions at 85%, 65%, and 25% MVC. The next set then commenced after a 2-min rest. For the five sets that involved FNS, the potentiated resting twitch was delivered immediately after the MVC.

Data analysis

In Studies 1-3, peak-to-peak amplitudes were measured for MEPs and M_{max}, and MEPs were normalized to M_{max}. Sizes of SITs were measured as the peak force after the stimulus subtracted from the voluntary force prior to the start of the SIT at ~25 ms after TMS and ~12 ms after FNS.

In Study 1, peak-to-peak amplitudes of the 3 M-waves at each stimulation intensity for the M-wave recruitment curve were averaged, as were the 3 H-reflexes at each stimulation intensity. Also, the resting twitch from the "optimal" TMS intensity in Study 1 was estimated using linear regression for each participant (36, 37). This regression included

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the 20 voluntary contractions performed by the participant at 50%, 70%, 85%, and 100% MVC (5 contractions per contraction intensity). In all 3 studies, peak-to-peak amplitudes of MEPs and sizes of SITs at each contraction intensity were averaged across the relevant number of test sets. In Study 3, voluntary activation from FNS was computed using the FNS SIT at 100% MVC and the FNS resting twitch. TMS voluntary activation was computed using the TMS SIT at 100% MVC and the resting twitch from FNS. We did not use the "best trial" or "best SIT" when computing voluntary activation for each individual. Instead, we used the mean of the relevant SITs.

Statistical analysis

In Study 1, a paired samples t-test and Cohen's *d* effect size for within-subjects design (d_z)were used to compare the size of the resting twitch from FNS and the size of the estimated resting twitch from TMS. One sample t-tests were used to compare amplitudes of VL and VM MEPs at 50% MVC to the criterion MEP amplitude at 50% MVC (i.e., 70% M_{max}). A one sample t-test was also used to compare the amplitude of HS MEPs to the criterion MEP amplitude at 100% MVC (i.e., 10% M_{max}). In Study 2, a paired samples t-test was used to compare the size of the FNS SIT and TMS SIT at 90% MVC. For this comparison, the TMS SIT was from the best of the five TMS intensities for each participant. In Study 3, paired samples t-tests were used to compare the size of the FNS SIT and TMS SIT at each contraction strength. Also, a paired samples t-test and Cohen's d_z effect size for within-subjects design were used to compare voluntary activation from FNS and the modified TMS procedure. SPSS version 25 (IBM, Armonk, United States) was used for the statistical analyses, except for effect sizes which were generated in RStudio (Version 1.3.1056, rstatix package). Statistical significance was set at $p \leq 0.05$. Data in the text and figures are presented as means (SD), unless noted otherwise.

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RESULTS

Study 1

M-waves and H-reflexes. Individual traces of HS M-waves and H-reflexes in two participants are depicted in Fig. 3*A*. The HS M-wave and H-reflex recruitment curves are depicted in Fig. 3*B*. HS H-reflexes were present in 10 of 12 participants. The group mean (n = 12) for HS M_{max} amplitude was 9.8 mV (SD 2.8; range: 6.8 – 15.2). The group means (n = 12) for VL and VM M_{max} amplitudes were 20.1 mV (SD 6.1; range: 11.7 – 31.9) and 18.2 mV (SD 5.2; range: 11.8 – 30.5), respectively.

MEPs. Amplitudes of VL and VM MEPs with the "optimal" TMS intensity are displayed in Fig. 4*A*. Amplitudes of HS MEPs are displayed in Fig. 4*B*. Notably, VL (51.4% M_{max} (SD 11.9), $p < 0.001$) and VM (54.2% M_{max} (SD 14.5), $p = 0.003$) MEPs at 50% MVC were below recommended guidelines for agonist muscles (≥70% VL M_{max}) for the group and importantly, for most individuals. While HS MEPs at 100% MVC (16.5% M_{max} (SD 10.3), $p = 0.052$) were not significantly above recommended guidelines for antagonist muscles (≤10% HS M_{max}) for the group of participants, they were above guidelines for more than half the individuals.

Resting twitch. For all participants, sizes of resting twitches of the knee extensors from FNS, and sizes of estimated resting twitches of the knee extensors from TMS, are displayed in Fig. 4*C*. The group mean (n = 12) for the estimated resting twitch from TMS (99.1 N (SD 37.2)) was smaller ($p < 0.001$, $d_z = 1.66$) than the resting twitch from FNS (142.4 N (SD 41.8)). Individual traces of SITs for one participant are displayed in Figure 5*A*. Figure 5*B* displays the regression used to estimate the resting twitch for the same participant.

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Study 2

M-waves. The group mean (n = 11) for HS M_{max} amplitude was 9.2 mV (SD 1.9; range: 6.6 – 12.3). The group means (n = 11) for VL and VM M_{max} amplitudes were 21.1 mV (SD 5.1; range: 14.2 – 27.7) and 20.4 mV (SD 5.8; range: 10.6 – 34), respectively.

MEPs and SITs. Amplitudes of VL and VM MEPs at 90% MVC with the five TMS intensities are displayed in Fig. 6*A*. Amplitudes of HS MEPs at 90% MVC are displayed in Fig. 6*B*. SITs at 90% MVC with FNS and the five TMS intensities are displayed in Fig. 6*C*. TMS 1 was the best intensity (i.e., the intensity that elicited the largest SITs at 90% MVC) in 4 participants. TMS 2, TMS 3, and TMS 4 were the best intensities for evoking SITs in two participants each. TMS 5 (the "optimal" TMS intensity) was the best for evoking SITs in one participant. When the best TMS intensity for each individual was considered (Fig. 6*D*), group means (n = 11) for the TMS SIT (16.1 N (SD 10.3)) and FNS SIT (20.9 N (SD 16.7)) at 90% MVC were not statistically different (p = 0.258). Also, when the best TMS intensity for each individual was considered, the group mean (n = 11) for the SIT ratio was 0.91 (SD 0.49; range: 0.12 – 1.53), with 4 participants (36%) exhibiting a SIT ratio <0.80 and 7 participants (64%) exhibiting a SIT ratio >0.80.

Study 3

M-waves. The group mean for HS M_{max} amplitude for participants (n = 8) who were eligible to complete the full experimental protocol was 14.5 mV (SD 6.2; range: 6.6 – 23.8). The group mean for VL and VM M_{max} were 21 mV (SD 8.2; range: 11.8 – 35.7) and 19.6 mV (SD 7.8; range: 4.8 – 27.2), respectively.

MEPs, SITs, voluntary activation. A flow diagram representing the decision process for participant eligibility based on evoked responses from SNS, FNS, and TMS is displayed in Fig. 7. Listed at the top of Table 2 are results from setup procedures for the 6 of 14 participants (42.8%) who were ineligible to undergo the full experimental protocol, as they

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exhibited SIT ratios <0.80 at 90% MVC during setup procedures. For these 6 participants, VL MEPs at 90% MVC were generally 20-40% VL M_{max} during setup procedures. HS MEPs were generally 5-20% HS M_{max}. The mean SIT ratio at 90% MVC was 0.46 (SD 0.18; range: 0.16-0.72). Table 2 also summarizes results from the 8 of 14 participants (57.2%) who were eligible to complete the full experiment, as they exhibited SIT ratios ≥0.80 at 90% MVC during setup procedures. For these 8 participants, VL MEPs at 90% MVC were generally 40-60% VL M_{max} during setup procedures. HS MEPs were generally 4-12% HS M_{max}. The mean SIT ratio at 90% MVC was 1.05 (SD 0.25; range 0.81-1.46).

Fig. 8 displays results from the experimental protocol in the eligible participants. Fig. 8*A* displays amplitudes of VL and VM MEPs. Fig. 8*B* displays amplitudes of HS MEPs. Fig. 8*C* displays TMS SITs and FNS SITs at 25-100% MVC. TMS SITs at 100% MVC (10.9 N (SD 6.4)) were statistically smaller ($p = 0.027$) than FNS SITs at 100% MVC (13.2 N (SD 6.7)). No statistically significant differences existed between TMS SITs and FNS SITs at other contraction intensities (all $p > 0.134$). Fig. 8*D* displays results of voluntary activation from FNS and the modified TMS procedure. Voluntary activation from the modified TMS procedure (87.3% (SD 7.1)) was statistically higher ($p = 0.030$, $d_z = 0.96$) than voluntary activation from FNS (84.5% (SD 7.6)).

DISCUSSION

Use of TMS to test voluntary activation of the knee extensors is common in research (4, 5, 8, 12, 14-16, 19, 23, 25, 26, 28-31, 33, 34, 40, 41). Yet, the validity of the technique has been uncertain. The current work clarifies this uncertainty. It illustrates that the contemporary TMS procedure is invalid for testing voluntary activation of the knee extensors. A modified version of the procedure, which combines TMS SITs at high contraction strengths with the resting twitch from FNS, improves the validity of TMS-based voluntary activation. However,

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449 it does so only for a small portion of individuals who meet eligibility criteria associated with
450 sizes of TMS SITs at 90% MVC in setup procedures.

451 **Study 1**

452 *Hamstrings M-waves.* HS M-waves were obtained reliably with electrical stimulation
453 of sciatic nerve branches. Prior to the current work, the validity of TMS for assessing
454 voluntary activation of the knee extensors was unclear because, for one, the degree to which
455 the TMS pulse might inadvertently activate the antagonist HS motor area (i.e., HS MEP / HS
456 M_{max}), and consequently reduce SITs of knee extensors, was unknown. As Table 1 illustrates,
457 several researchers have reported raw MEPs from biceps femoris, but only Sidhu et al.
458 attempted to normalize MEPs to M_{max} (28). Their technique involved high electrical currents
459 (~650 mA) delivered to the sciatic nerve but was never widely adopted because the technique
460 is difficult to perform and the stimulation intensity is found intolerable by most participants
461 (28). In the current series of studies, HS M-waves were evoked with a much lower
462 stimulation intensity (160 – 340 mA), and only 2 of 34 participants (6%) withdrew due to
463 discomfort from SNS. Moreover, HS M-waves were obtained c in 29 of the 32 participants
464 (91%) who found SNS tolerable. H-reflexes were noted in most participants during M-wave
465 stimulation. This incidental finding is reported for its potential interest for future
466 neurophysiological assessments of HS and to clarify the waveform recorded after SNS.

467 A few caveats of SNS require discussion. First, joint angle and muscle length impact
468 amplitudes of M-waves (11, 18). Thus, results from the M-wave and H-reflex recruitment
469 curves in the current experiments are specific to the seated posture studied. Second, as a
470 result of the seated posture and the relaxed state in which M-waves were evoked, the muscles
471 and adipose tissue of the posterior thigh naturally sagged. Consequently, in pilot testing,
472 surface electrodes placed on the skin were inadequate for delivering SNS. The most effective
473 method for delivering SNS involved the investigator pushing up on the sagging muscle with a

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12-mm diameter probe that resembled a pen. Third, the optimal stimulation position for SNS was not the same for all participants. Fourth, in some instances, movement of the probe a few millimeters from the optimal position caused marked changes in the M-wave. Large stimulus artifact sometimes dominated the EMG recording and prevented M-wave measurement when the probe was not positioned optimally. Nevertheless, in most participants, clear M-waves were observed if the probe was positioned at a site on the lateral or mid-lateral aspect of the posterior thigh, about halfway along the length of the femur. Finally, inter-electrode spacing also impacts M-wave area and amplitude (38). The distance between HS recording electrodes in the current research was ~40 mm.

HS, VL, VM MEPs and the resting twitch. Reliable acquisition of HS M_{max} permitted examination of the degree to which the "optimal" TMS intensity might inadvertently activate antagonist HS motoneurons. This intensity was considered "optimal" because we attempted to adhere to recommended guidelines when identifying it although strict adherence was not possible. The guidelines state TMS intensities for assessment of voluntary activation should evoke large agonist MEPs at 50% MVC (≥70% VL M_{max}) and small antagonist MEPs at 100% MVC (≤10% HS M_{max}) (22). We found that the "optimal" TMS intensity evoked agonist VL MEPs at 50% MVC and antagonist HS MEPs at 100% MVC that did not meet these guidelines for twelve of twelve and seven of twelve participants, respectively. Consequently, the estimated resting twitch from TMS was markedly and consistently smaller than the actual resting twitch from FNS. This is problematic because SITs of the knee extensors evoked by TMS and FNS are expected to be similar, as both stimulations activate all knee extensor muscles. In contrast, in the elbow flexors, TMS and electrical stimulation of the musculocutaneous nerve are not expected to evoke similar SITs because the nerve stimulation does not activate brachioradialis (3). Thus, results from Study 1 illustrate that the

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498 contemporary TMS procedure for assessment of voluntary activation is invalid when applied
499 to the knee extensors.

500 **Study 2**

501 In Studies 2 and 3, we abandoned the idea that a resting twitch could be estimated
502 using the contemporary TMS procedure, which is valid in other muscle groups (e.g., elbow
503 flexors) (36, 37). Instead, we thought that if TMS evokes adequate SITs at high contractions
504 strengths, these SITs could be combined with the resting twitch from FNS to compute
505 voluntary activation. The physiological rationale behind this approach is that a smaller-than-
506 optimal agonist MEP may still give an adequate SIT at high contraction strengths because
507 any motoneurons not firing voluntarily at the time of stimulation are likely to be recruited
508 into the MEP. These motoneurons should be in the subliminal fringe because of the strong
509 excitatory drive associated with the voluntary contraction and then provide the biggest
510 increments in force because they are not contributing already. Therefore, in Study 2, our
511 primary aim was to determine if TMS could evoke SITs at 90% MVC that are comparable to
512 FNS SITs at 90% MVC. Moreover, we tested five TMS intensities, with the "optimal" TMS
513 intensity serving as the highest intensity. We tested TMS intensities lower than "optimal"
514 because in Study 1, the "optimal" intensity evoked antagonist HS MEPs that were too big.

515 In Study 2, we found that the TMS intensity that evoked the largest SITs at 90%
516 MVC (i.e., the "best" TMS intensity) differed between participants. "TMS 1" was the best
517 TMS intensity for 4 of the 11 participants. "TMS 5" (the "optimal" TMS intensity) was the
518 best intensity for only one participant. We defined an adequate TMS/FNS SIT ratio as ≥0.80.
519 When the best TMS intensity for each participant was considered, a SIT ratio ≥0.80 was
520 exhibited in 64% of participants. In our post-hoc evaluations, we noted VL and VM MEPs
521 could be as low as 40% M_{max} and HS MEPs as high as 15% M_{max}, at 90% MVC and still
522 result in a SIT ratio ≥0.80. Therefore, our aim in Study 3 was to determine if this post-hoc

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information could be incorporated into eligibility criteria to identify individuals in whom the modified TMS technique might provide a valid measure of voluntary activation.

Study 3

In Study 3, TMS SITs (10.9 N) were smaller than FNS SITs (13.2 N) at 100% MVC – the condition under which voluntary activation was assessed. Consequently, the modified TMS procedure overestimated voluntary activation (87.3%) compared to FNS (84.5%). Thus, whereas SITs at 90% MVC in Study 2 were comparable with TMS and FNS, forming the basis for the TMS eligibility criteria in Study 3, the SITs at 100% MVC were different when evoked from these two types of stimulation. Yet, the modified TMS procedure was valid for measuring voluntary activation in 5 of 8 participants (Table 2), if validity is defined post-hoc as a raw difference of <3% between the TMS and FNS procedures. Notably, in the 3 participants in whom TMS did not provide a valid measure of activation (participants A7, A9, A10), VL and VM MEPs at 100% MVC were generally lower, and HS MEPs at 100% MVC generally higher, than in the other participants. Both A9 and A10 exhibited VL MEPs at 100% MVC that were <40% M_{max}, and A7 and A9 exhibited HS MEPs at 100% MVC that were ≥20% M_{max}. Moreover, participants A7, A9, and A10 exhibited the smallest VL MEPs (all <50% M_{max}) during the setup procedures at 90% MVC. Thus, the use of the SIT ratio at 90% MVC in setup procedures was only partly effective at identifying individuals in whom TMS provides a valid measure of activation. Because a total of 21 individuals attended the laboratory for Study 3, and only 5 made it through TMS eligibility testing *and* exhibited adequate TMS SITs at 100% MVC, the modified TMS procedure is only useful in about 1 of every 4 healthy young adults who attend the laboratory.

Should TMS be used to measure voluntary activation of the knee extensors?

Based on results from Study 1, we recommend the contemporary TMS procedure for assessing voluntary activation of the knee extensors be abandoned. It does not provide an

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accurate estimate of the resting twitch. The modified TMS procedure in Study 3 can be used, but only when rigorous eligibility criteria are applied. The modified technique should only be used in individuals who, in setup procedures at 90% MVC, exhibit (a) TMS/FNS SIT ratio ≥0.80, (b) VL MEPs ≥50% M_{max}, and (c) HS MEPs <15% M_{max}. In Table 4, we summarize steps necessary to determine participant eligibility for examination of voluntary activation of the knee extensors with the modified TMS procedure. The purpose of testing SITs at 90% MVC rather than 100% MVC in setup procedures is to guarantee that a measurable SIT should occur in all contractions and to help mitigate the effects of strong contractions on fatigue and subsequent tests.

Each investigator will need to decide if information gained from TMS-based voluntary activation of the knee extensors is worth the time and resources needed to conduct the procedure. The primary benefit of TMS-based voluntary activation is that it can test if descending drive (i.e., voluntary output) from the cortex is suboptimal during MVCs. Such information might be useful in understanding the anatomical locations and physiological processes associated with maintained or compromised neural drive from exercise, aging, or neurological impairment. However, TMS-based voluntary activation of the knee extensors has drawbacks. First, SNS will have to be added to experimental protocols to confirm the validity of the TMS procedure in the knee extensors. The ratio of HS MEPs to HS M_{max} will be needed to determine if the TMS pulse activates too much of the antagonist HS in each participant. Addition of SNS to study protocols will make the protocols longer; however, only HS M_{max}, rather than the entire HS H/M-wave recruitment curve, is needed to determine TMS validity. Second, as is sometimes the case with stimulation procedures, some participants might withdraw due to stimulation discomfort or they might be ineligible due to inadequate responses to stimulation. For example, in Study 3, of the 21 individuals who attended the laboratory, HS M-waves could not be obtained in two of them (9.5% of original

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sample), five withdrew due to discomfort from SNS, FNS, or TMS (23.8% of original sample), six were deemed ineligible based on their TMS/FNS SIT ratio (28.6% of original sample), and of the remaining 8 who underwent complete testing, post-hoc analysis revealed the modified TMS was probably invalid for measuring voluntary activation in three of them (14.3% of original sample) (Table 2). Thus, of the 21 individuals who attended the laboratory, only 5 (23.8% of original sample) completed the full protocol and had valid responses. Thus, an investigator who seeks to use the modified procedure for TMS-based voluntary activation to examine the effects of fatiguing exercise on descending neural drive from the cortex will likely need to screen a large number of individuals to obtain an adequate, final sample size. Finally, our studies consisted mostly of healthy young adults who were recruited from local universities. It is unknown if similar proportions of individuals would be deemed eligible/ineligible in clinical populations and other groups.

Conclusion

The current studies examined if TMS is a valid tool for assessing voluntary activation of the knee extensors. We developed a method for evoking HS M-waves reliably in most individuals. This permitted a more complete examination of the validity of TMS-based voluntary activation of the knee extensors. The "optimal" TMS intensity, which was based on contemporary guidelines for testing voluntary activation with TMS, led to (a) activation of too few agonist quadriceps motoneurons, (b) activation of too many antagonist hamstring motoneurons, and (c) underestimation of the size of the resting twitch. Consequently, the contemporary TMS procedure for assessing voluntary activation is invalid when applied to the knee extensors. A modified version of the procedure, which combines TMS SITs at high contraction strengths with resting twitches from FNS accurately measured voluntary activation, but only in some individuals who met rigorous inclusion criteria for sizes of SITs and MEPs at 90% MVC.

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DISCLOSURES

All authors affirm there are no competing interests, financial or otherwise.

AUTHOR CONTRIBUTIONS

JLN, DSK, HTF, and JLT conceived and designed the research; JLN, DSK, HTF, and JLT performed experiments; JLN analyzed data; JLN, DSK, HTF, and JLT interpreted results; JLN drafted the manuscript and figures; JLN, DSK, HTF, and JLT edited and revised the manuscript; JLN, DSK, HTF, CL, and JLT approved the final version of the manuscript.

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726 **Figure captions**

727 **Fig. 1.** Experimental setup for Studies 1-3. *A*: Testing was conducted on the right leg.
728 Depicted are positions of the stimulating electrodes for electrical stimulation of the femoral
729 nerve (FNS) and sciatic nerve branches (SNS). The gap between the seat and knee bar
730 allowed for SNS with a cathode probe, which was held in the hand of the investigator. On top
731 of the knee bar was a foam pad. A section of the foam was cut out to allow for placement of
732 recording electrodes on medial hamstrings (HS). Recording electrodes for vastus lateralis
733 (VL) are also depicted. *B*: Posterior view of the right thigh. Depicted are positions of the
734 stimulating and recording electrodes for SNS. The optimal position of the cathode probe for
735 evoking HS M-waves was typically on the lateral or mid-lateral aspect of the posterior thigh,
736 about halfway along the length of the femur. The optimal position was usually within the area
737 depicted by the dotted box. Recording electrodes were placed on the distal portion of the HS
738 and likely recorded from both semitendinosus (ST) and semimembranosus (SM). No
739 recordings were made from biceps femoris (BF).

740 **Fig. 2.** Experimental protocols for Studies 1-3. Each study consisted of a single testing
741 session. For Study 1, setup procedures and measurements included: electrical stimulation of
742 sciatic nerve branches (SNS) to evoke the maximal compound muscle action potential (M_{max})
743 for the medial hamstrings (HS) and to obtain the HS M-wave and H-reflex recruitment curve;
744 electrical stimulation of the femoral nerve (FNS) to evoke M_{max} for vastus lateralis (VL) and
745 vastus medialis (VM); maximal voluntary contractions (MVC) of the knee extensors; and
746 setup procedures for determining the "optimal" intensity for transcranial magnetic stimulation
747 (TMS). The experimental protocol tested the effect of the "optimal" TMS intensity (69% (SD
748 5)) on superimposed twitches (SIT) of the knee extensors and motor evoked potentials (MEP)
749 of VL, VM, and HS during 5 sets of brief isometric contractions of the knee extensors. The
750 first part of a given set consisted of TMS-evoked responses at 100% MVC, 70% MVC, and

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25% MVC, with the potentiated resting twitch (RT) from FNS occurring immediately after the contraction at 100% MVC. The second part of the set, which occurred after a 2-min rest, consisted of TMS-evoked responses at 85% and 50% MVC. Study 2 included many of the same measurements and procedures as Study 1. After establishing active motor threshold (AMT) and the "optimal" TMS intensity, the effect of five different TMS intensities on SITs and MEPs was assessed in 18 sets of brief isometric contractions of the knee extensors at 90% and 50% MVC. Three sets were completed with FNS and three were completed with each of five TMS intensities ("TMS 1": 43% (SD 6); "TMS 2": 52% (SD 6); "TMS 3": 58% (SD 7); "TMS 4": 63% (SD 7); and "TMS 5": 68% (SD 8)). Study 3 included many of the same measurements and procedures as Studies 1 and 2. After the setup procedures and the identification of eligible individuals based on sizes of SITs and MEPs, the effect of TMS (56% (SD 18) on SITs and MEPs was assessed in 10 sets of brief isometric contractions of the knee extensors. Five sets involved use of the "high SIT ratio" TMS intensity, whereas the other five sets involved use of FNS. The first part of a given set consisted of evoked responses at 100% MVC, 75% and 25% MVC. The second part of the set, which occurred after a 2-min rest, consisted of evoked responses during contractions at 85%, 65%, and 25% MVC. For the five sets that involved FNS, the potentiated RT was delivered immediately after the contraction at 100% MVC.

Fig. 3. M-waves and H-reflexes of the medial hamstrings (HS) in Study 1. *A*: Traces of HS M-waves and H-reflexes from 2 participants. M-waves on the left for the 2 participants occurred with a stimulation intensity of 0.7x the minimal intensity necessary to evoke HS M_{max}. M-waves on the right for the 2 participants are M_{max} and occurred at stimulation intensities of 1.3x the minimal intensity necessary to evoke HS M_{max}. For traces on the left, the first waveform is an HS M-wave. The second waveform is an HS H-reflex. For traces on the right, the first waveform is HS M_{max}. No H-reflexes were present, as expected at the high

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776 stimulation intensity. Note that stimulus artifact was problematic in recording HS M_{max} and
777 almost obscures the M-wave in Participant 2. *B*: Recruitment curves for HS M-waves (filled
778 circles, $n = 12$) and HS H-reflexes (filled triangles, $n = 10$). H-reflexes were not present in 2
779 of the 12 participants. Data are means (SD).

780 **Fig. 4.** Results from Study 1. *A:* Effect of the "optimal" transcranial magnetic stimulation
781 (TMS) intensity on amplitudes of vastus lateralis (VL) and vastus medialis (VM) motor
782 evoked potentials (MEP) during brief isometric knee extensor contractions at 25-100%
783 maximal voluntary contraction (MVC). Amplitudes are expressed as a percentage of the
784 maximal compound muscle action potential (M_{max}). Individual participants are represented by
785 circles. Group means ($n = 12$) are represented by solid black dashes. The recommended size
786 of agonist MEPs (≥70% M_{max}) for tests of voluntary activation with TMS is represented by
787 the dashed horizontal line. *Indicates a statistically significant difference between VL and
788 VM MEPs at 50% MVC compared to the recommended VL and VM MEP sizes. *B:* Effect of
789 the "optimal" TMS intensity on amplitudes of medial hamstrings (HS) MEPs during brief
790 isometric knee extensor contractions at 25-100% MVC. Individual participants are
791 represented by triangles. Group means ($n = 12$) are represented by solid black dashes. The
792 recommended size of antagonist MEPs (<10% M_{max}) for tests of voluntary activation with
793 TMS is represented by the dashed horizontal line. *C:* Actual resting twitch forces (Newtons,
794 N) from femoral nervous stimulation (FNS) and the estimated resting twitch forces from the
795 TMS procedure with the "optimal" TMS intensity. Circles represent individual participants.
796 Each individual's actual and estimated resting twitches are connected by a line. Note that
797 estimated resting twitches (TMS optimal) are systematically smaller than actual (FNS)
798 resting twitches. Group means ($n = 12$) are represented by solid black dashes. *Indicates
799 statistically significant difference between TMS optimal and FNS.

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Fig. 5. An individual participant's superimposed twitches (SITs) evoked by the "optimal" intensity of transcranial magnetic stimulation (TMS). *A*: Raw traces of SITs across the five contraction intensities (25%, 50%, 70%, 85%, and 100% MVC). The dashed arrow (i.e., 0 sec) indicates when the TMS pulse was delivered. The solid arrow indicates the peak of the SIT. The vertical dotted line indicates the approximate time at which the baseline force for the SIT measurement was taken (~25 ms after TMS pulse was delivered). The SIT was calculated as the difference between the peak of the SIT and the baseline force. NB. Due to an administrative error, this participant performed six rather than five voluntary contractions at 100% MVC and four rather than five voluntary contractions at 85% MVC. *B*: Regression used to estimate the resting twitch from TMS in the same participant. NB. The voluntary contractions at 25% MVC are not included when estimating the resting twitch from TMS.

Fig. 6. Results from Study 2. *A:* Effect of five transcranial magnetic stimulation (TMS) intensities on amplitudes of vastus lateralis (VL) and vastus medialis (VM) motor evoked potentials (MEP) during brief isometric contractions of the knee extensors at 90% maximal voluntary contraction (MVC). "TMS 1" is the lowest TMS intensity (43% (SD 6)). "TMS 5" – the "optimal" intensity – is the highest TMS intensity (63% (SD 8)). Amplitudes of MEPs are expressed as a percentage of the maximal compound muscle action potential (M_{max}). Individual participants are represented by circles. Group means (n = 11) are represented by black dashes. *B:* Effect of five TMS intensities on amplitudes of medial hamstrings (HS) MEPs during brief isometric contractions of the knee extensors at 90% MVC. The dashed horizontal line represents the recommended size of antagonist MEPs for tests of voluntary activation with TMS (<10% M_{max}). Individual participants are represented by triangles. Group means (n = 11) are represented by solid black dashes. *C:* Effect of the five TMS intensities and femoral nerve stimulation (FNS) on superimposed twitch (SIT) forces (Newtons, N) during brief isometric contractions of the knee extensors at 90% MVC.

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825 Individual participants are represented by circles. To illustrate the variation between
826 individuals, 1 participant is represented by an open square. For this participant, the SIT from
827 FNS (36 N) was markedly larger than SITs from the five TMS intensities (11-18 N). The best
828 TMS intensity for this participant (i.e. the one that elicited the largest SIT) was TMS 5 (18
829 N). Another participant is represented by an X. For this participant, the SIT from FNS (8.8 N)
830 was not different from SITs with the five TMS intensities (7-10 N). The best TMS intensity
831 for this participant was TMS 2 (10 N). Group means (n = 11) are represented by solid black
832 dashes. *D:* SITs during brief isometric contractions of the knee extensors at 90% MVC with
833 FNS and the TMS intensity that evoked the largest SIT in each participant (i.e., "TMS best").
834 Individual participants are represented by circles. Each individual's data are connected by a
835 line. For some participants, TMS of a particular intensity evoked SITs that were comparable
836 in size, or larger in size, than SITs from FNS. Group means (n = 11) are represented by solid
837 black dashes. Data displayed for SITs from FNS in this figure are the same as displayed in
838 6*C*.

839 **Fig. 7.** Flow diagram of steps used in Study 3 to determine participant eligibility. After
840 assessments for hamstrings and quadriceps M_{max}, eligibility was based on sizes of evoked
841 responses from transcranial magnetic stimulation (TMS). Eight participants were deemed
842 eligible; 6 were ineligible. Two participants were ineligible because their superimposed
843 twitch (SIT) ratio was too small (<0.80), their vastus lateralis (VL) motor evoked potentials
844 (MEP) were too small (<40% VL M_{max}), and their medial hamstrings (HS) MEPs were too
845 big (>15% HS M_{max}). Three participants were ineligible because their SIT ratio and VL
846 MEPs were too small. One participant was ineligible because their SIT ratio was too small.
847 Of the 8 eligible participants who underwent all tests, post-hoc examination of their data
848 revealed TMS was a valid tool for assessing voluntary activation in 5 of them and invalid in
849 the other 3. Validity was arbitrarily defined as a raw difference of <3% between voluntary

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activation from FNS and TMS (i.e., the "high SIT ratio" TMS intensity). See Table 2 for individual data of evoked responses in eligible and ineligible participants.

Fig. 8. Results from Study 3. *A:* Effect of the "high SIT ratio" transcranial magnetic stimulation (TMS) intensity on amplitudes of vastus lateralis (VL) and vastus medialis (VM) motor evoked potentials (MEP) during brief isometric knee extensor contractions at 25-100% maximal voluntary contraction (MVC). Amplitudes are expressed as a percentage of the maximal compound muscle action potential (M_{max}). Individual participants (n = 8) are represented by circles. Group means are represented by solid black dashes. The adjusted criterion size for agonist MEPs (≥40% M_{max}) for tests of voluntary activation with TMS (based on Study 2) is represented by the dashed horizontal line. *B:* Effect of the "high SIT ratio" TMS intensity on amplitudes of medial hamstrings (HS) MEPs during brief isometric knee extensor contractions at 25-100% MVC. Individual participants (n = 8) are represented by triangles. Group means are represented by solid black dashes. The adjusted criterion size for antagonist MEPs (≤15% M_{max}) for tests of voluntary activation with TMS (based on Study 2) is represented by the dashed horizontal line. *C:* Effect of the "high SIT ratio" TMS intensity (filled circles) and femoral nerve stimulation (FNS) (open circles) on superimposed twitch forces (Newtons, N) during brief isometric knee extensor contractions at 25-100% MVC. Individual participants (n = 8) are represented by circles. Each individual's data are connected by a line. Group means are represented by solid black dashes. *Indicates statistically significant difference between TMS and FNS. *D:* Voluntary activation of the knee extensors as assessed by TMS (filled circles) and FNS (open circles). Individual participants (n = 8) are represented by circles. Each individual's data are connected by a line. Group means are represented by solid black dashes. *Indicates statistically significant difference between TMS and FNS.

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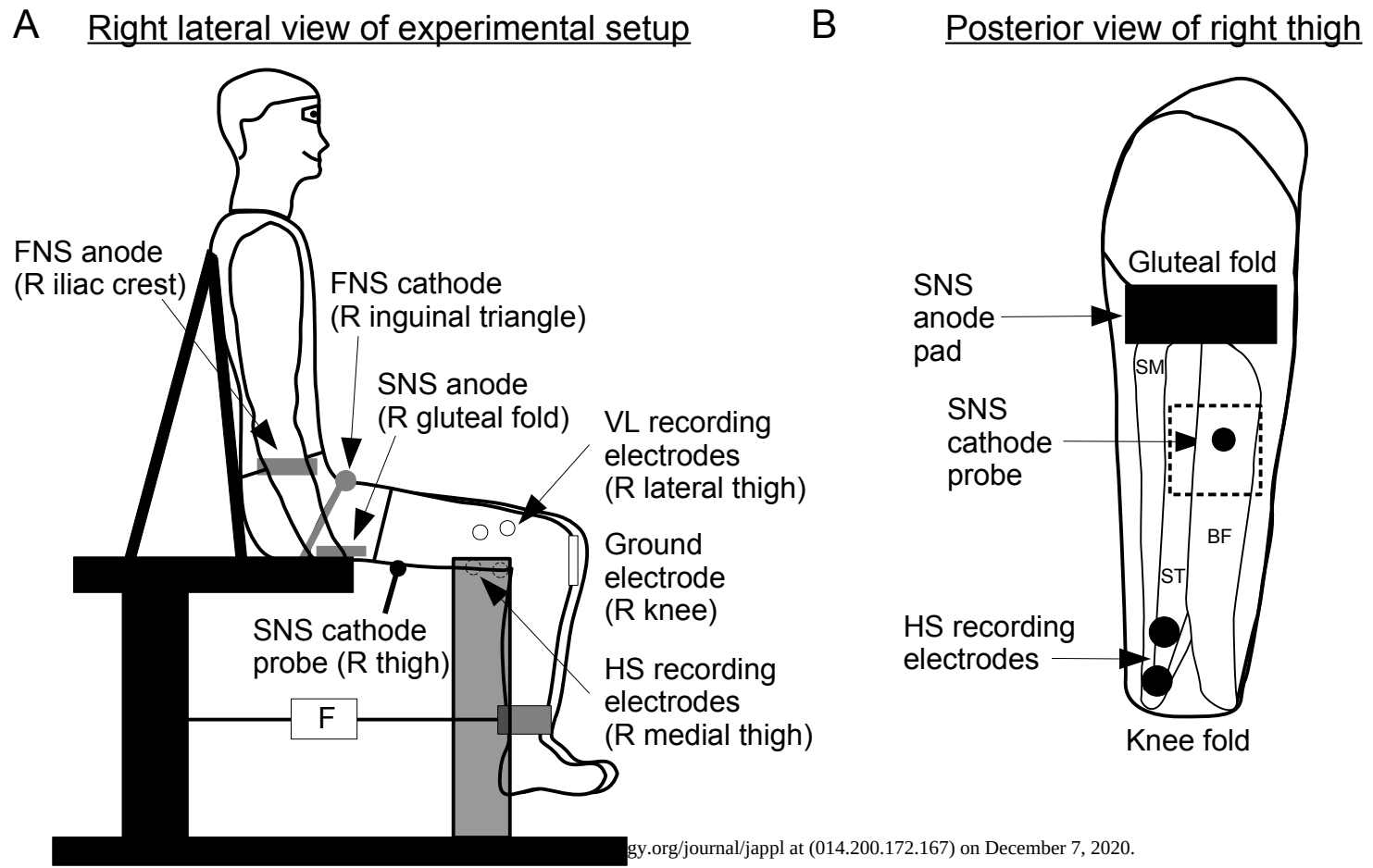

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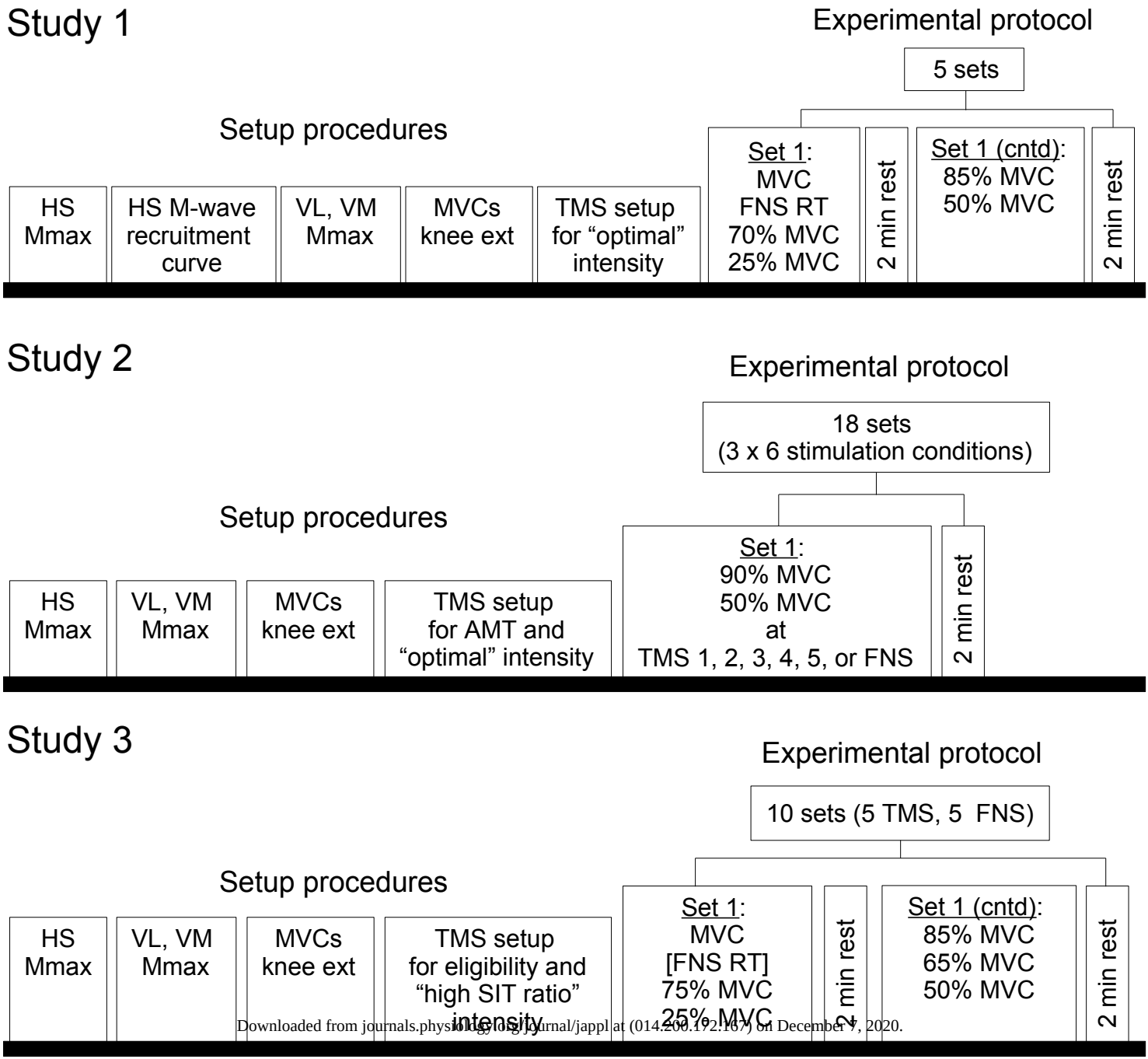

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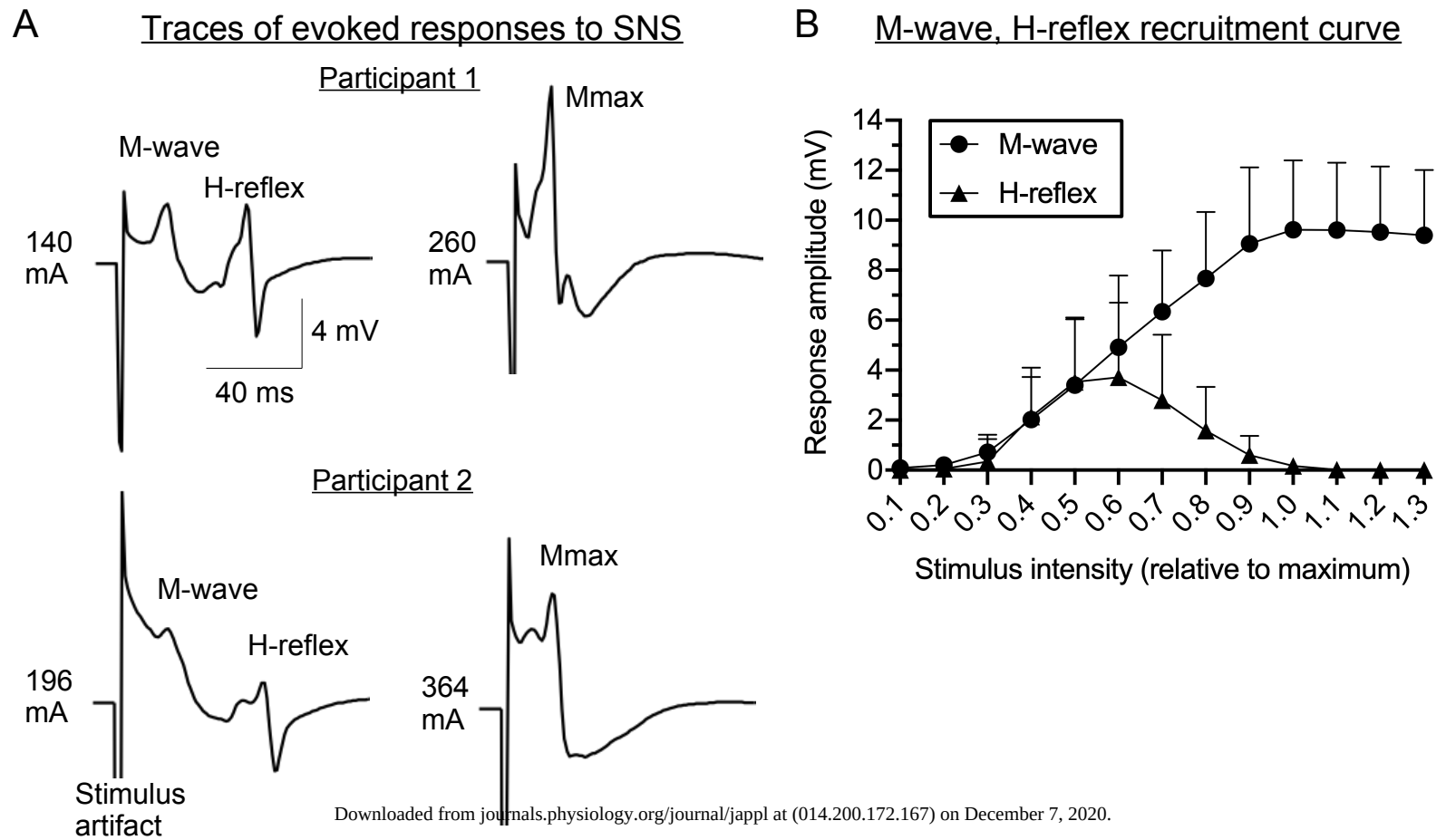

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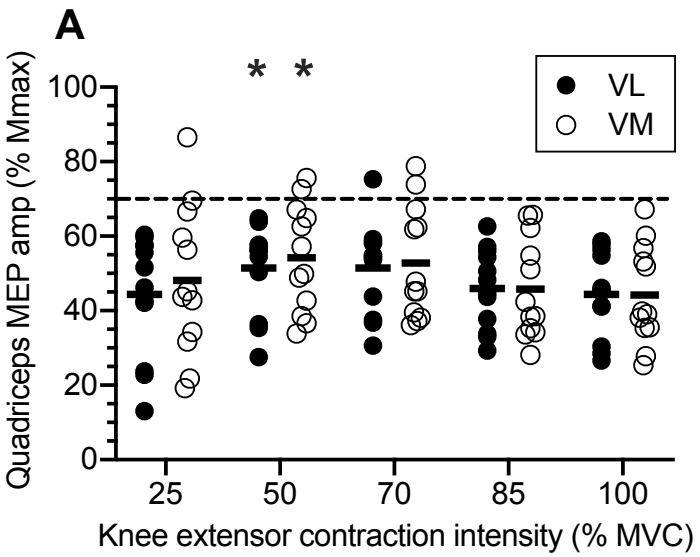

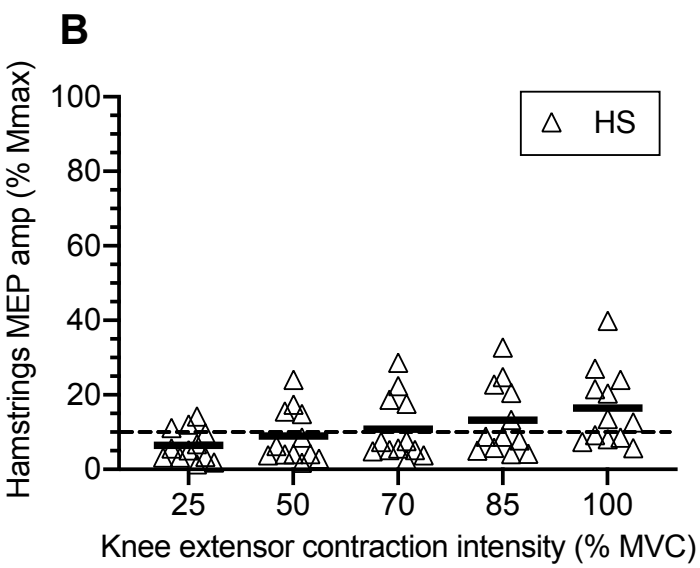

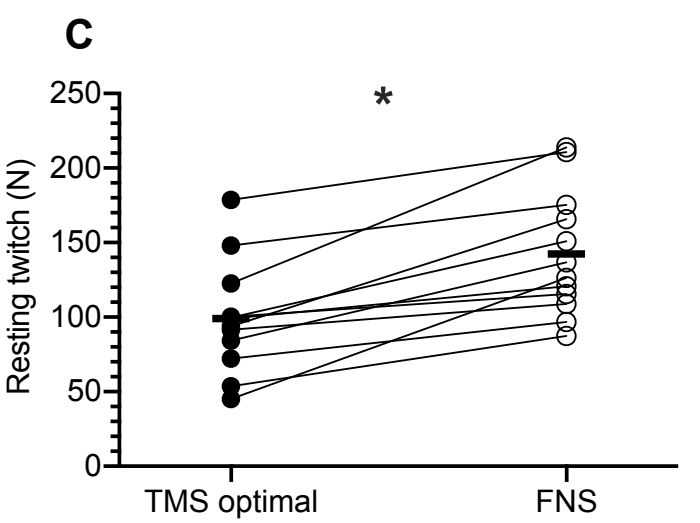

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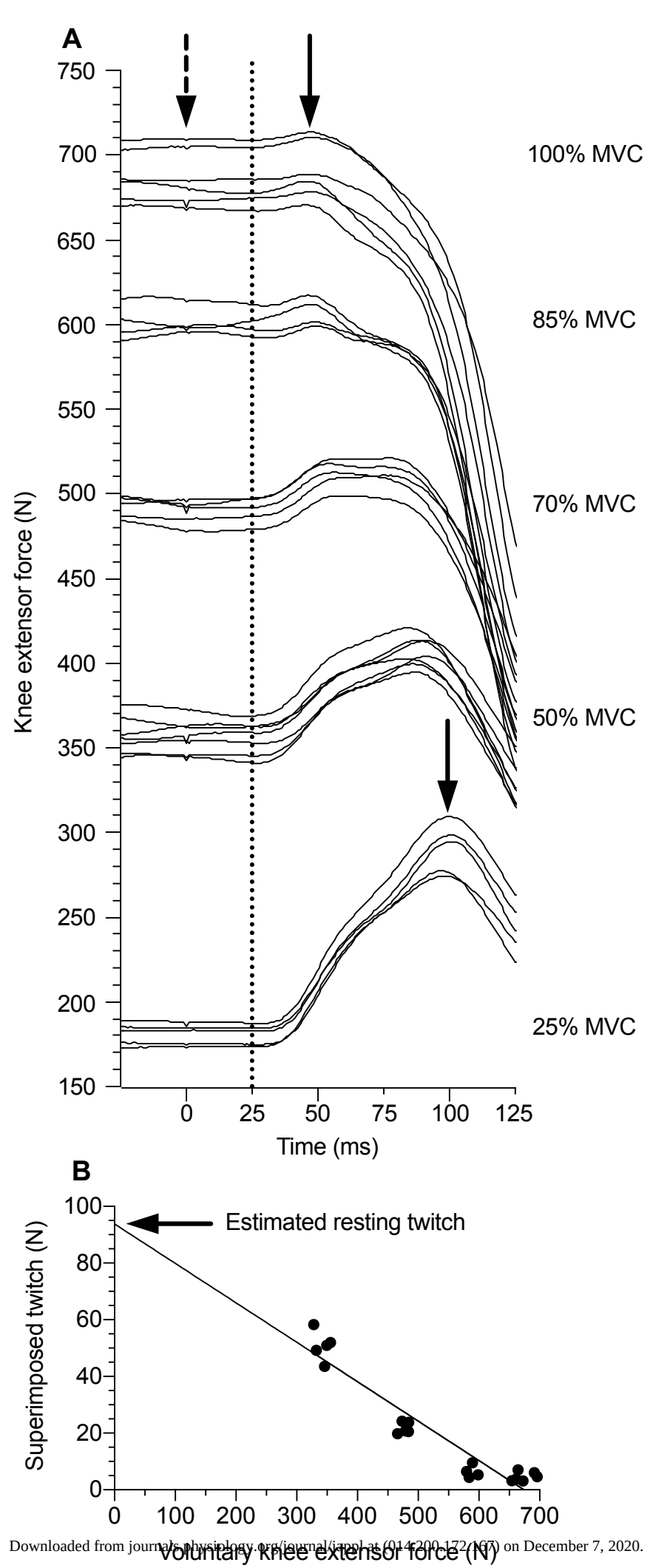

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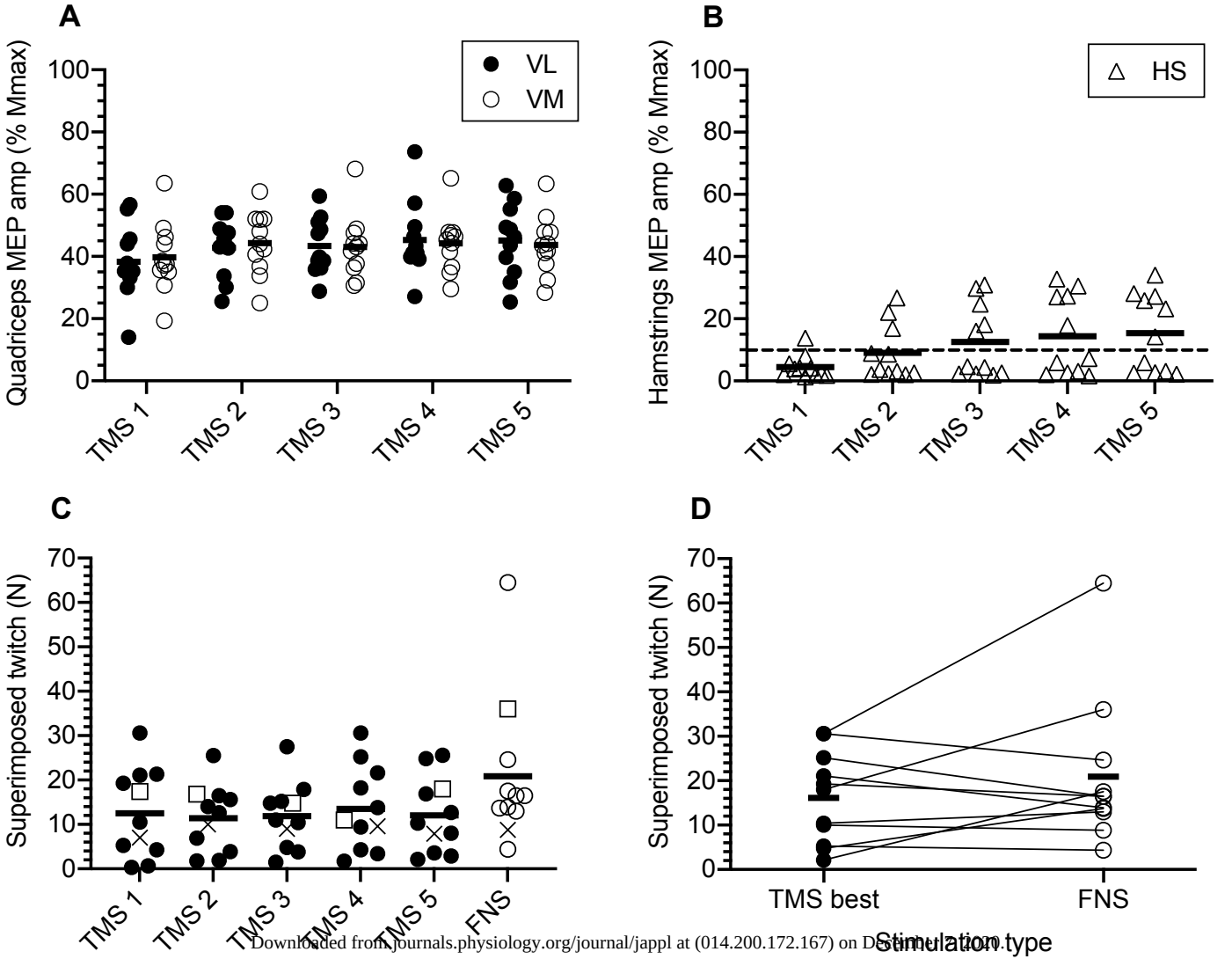

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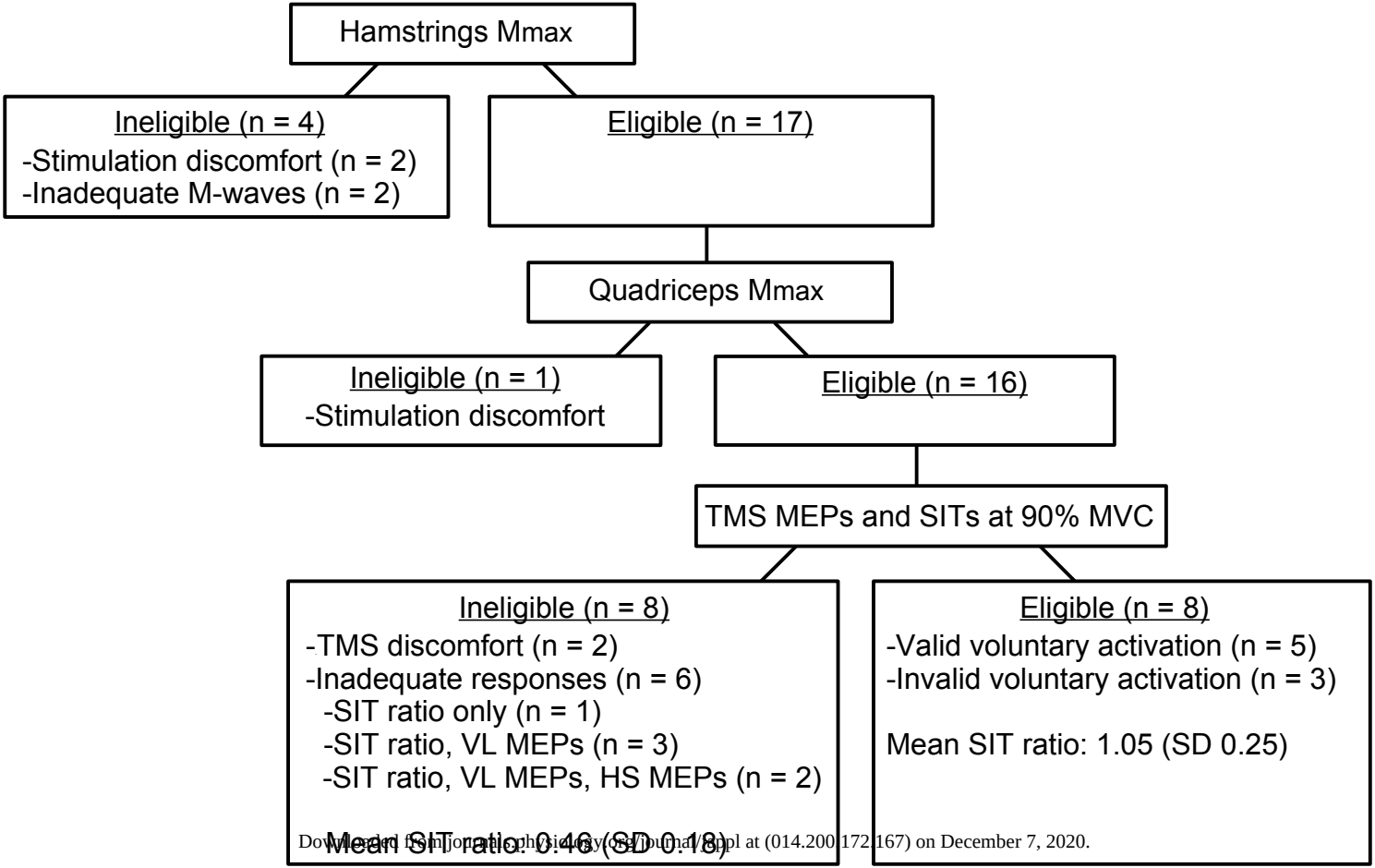

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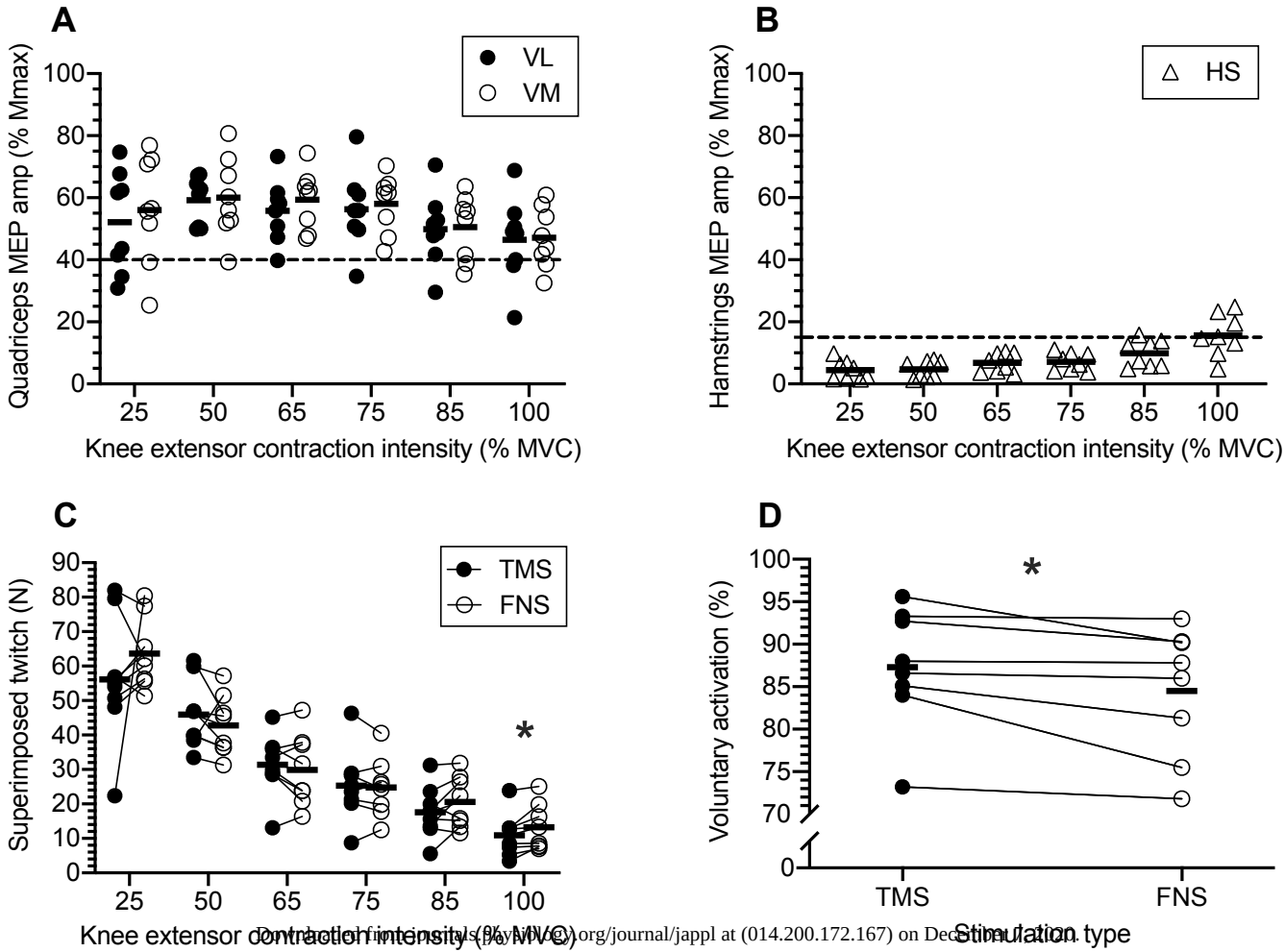

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Table 1. Methods that have been used to determine intensity of transcranial magnetic stimulation (TMS) to test voluntary activation of the knee extensors.

Study	TMS device	Coil type	BF M_{max} measured?	MEPs measured	TMS intensity
Arnal et al. (4)	Magstim 200[2]	Cone	No	BF, VL	TMS intensity (mean: 65-69% output) was lowest intensity that evoked maximal VL MEP at 20% MVC. SITs and VL and BF MEPs considered when coil position was determined with 50% output at 10% MVC.
Bachasson et al. (5)	Magstim 200[2]	Cone	No	BF, VL, VM, RF	TMS intensity (30-100%) set to evoke largest VL MEP at 20% MVC. Coil positioned to evoke largest SITs and VM, VL, RF MEPs, with small BF MEP (<10% "raw quadriceps MEP amplitude") at 10% MVC.
Bowtell et al. (8)	Magstim 200	Cone	No	BF, VM	TMS intensity was 120% of AMT (mean: 67% output). AMT based on VL MEPs at 5% MVC.
Dekerle et al. (9)	Magstim 200	Cone	No	BF, VL	TMS intensity (mean: 66% output) set to evoke largest SIT at 50% MVC. VL and BF MEPs were considered for coil position at 20% MVC.
Dekerle et al. (10)	Magstim 200	Cone	No	BF, VL	TMS intensity (mean: 65% output) set to evoke largest SIT at 50% MVC. VL and BF MEPs were considered for coil position at 20% MVC.
Fernandez del Olmo et al. (12)	Magstim 200[2]	Figure 8	No	BF, RF	TMS intensity (75-95% output) set to evoke RF MEPs of ~90% M_{max} at 50% MVC. RF and BF MEPs were considered for coil position.
Goodall et al. (15)	Magstim 200	Cone	No	BF, VL	TMS intensity was 130% of RMT (mean: 75% output). Intensity deemed appropriate as it evoked VL MEPs of 80-100% of VL M_{max} at ≥50% MVCs and evoked "only a small MEP" in BF.
Goodall et al. (16)	Magstim 200	Cone	No	BF, VL	TMS intensity was 130% of RMT (mean: 73%). Intensity deemed appropriate as it evoked VL MEPs of 60-100% M_{max} at ≥50% MVCs, while it evoked BF MEPs of ~20% the size of VL MEPs.
Goodall et al. (14)	Magstim 200	Cone	No	BF, VL	TMS intensity was 130% of RMT (mean: 73% output). RMT based on VL MEPs. This intensity was deemed appropriate as it evoked VL MEPs of 72% M_{max} at 100% MVC and BF MEPs 8% the size of VL MEPs.
Marillier et al. (19)	Magstim 200	Cone	No	BF, VL, VM, RF	TMS intensity was 140% AMT (mean: ~63% output). AMT based on MEPs at 10% MVC. Coil position determined with 60% output at 10% MVC and aimed to maximize VM, VL, and RF MEPs, while minimizing BF MEPs (<10% of "maximal quadriceps muscle M-wave").
Pageaux et al. (23)	Magstim 200[2]	Cone	No	BF, VL, VM, RF	TMS intensity (mean: 56% output) set to evoke largest VL MEP and a small BF MEP at 50% MVC.
Rupp et al. (25)	Magstim 200	Cone	No	BF, VL, VM, RF	TMS intensity set to evoke largest RF MEP and small BF MEP (<10% of RF M_{max}) at 50% MVC.
Senefeld et al. (26)	Magstim 200	Cone	No	VL, VM, RF	TMS intensity set to evoke maximal SITs and RF MEPs at 50% MVC.
Sidhu et al. (28)	Magstim 200[2]	Cone	Yes	BF, RF	TMS intensity (range: 40%–60% output) set to evoke largest RF MEP (at least 50% Mmax) and small BF MEP (<10% of raw quadriceps MEP amplitude) at 50% MVC.
Sidhu et al. (29)	Magstim 200[2]	Cone	No	BF, RF	TMS intensity set to evoke largest RF MEP (~90% RF M_{max}) and a small BF MEP (<10% of raw quadriceps MEP) at 50% MVC.
Souron et al. (30)	Magstim 200[2]	Cone	No	BF, VL, RF	TMS intensity (mean: 66% output) set to evoke largest VL MEP, RF MEP, and SIT, and small BF MEP at 20% MVC.
Sundberg et al. (31)	Magstim 200[2]	Cone	No	VL, VM, RF	TMS intensity set to evoke largest SIT and VL MEP at 40% MVC.
Temesi et al. (33)	Magstim 200[2]	Cone	No	BF, VL, VM, RF	Various TMS methods and intensities used.
Temesi et al. (34)	Magstim 200[2]	Cone	No	VL	TMS intensity (mean: 66% output) set to evoke the "largest MEP amplitudes" at 20% MVC.
Zghal et al. (40)	Magstim 200	Single	No	BF, VL, VM, RF	TMS intensity set to evoke largest RF MEP with a small BF MEP (<10% of RF M_{max}) at 50% MVC.
Zghal et al. (41)	Magstim 200[2]	Single	No	BF, VL, VM, RF	TMS intensity set to evoke largest RF MEP with a small BF MEP (<10% of RF M_{max}) at 50% MVC.

AMT = active motor threshold; BF = biceps femoris; M_{max} = maximal compound muscle action potential; RF = rectus femoris; RMT = resting motor threshold; TMS = transcranial magnetic stimulation; VM = vastus medialis; VL = vastus lateralis

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Table 2. Data from eligible and ineligible participants in Study 3.

Participant	Setup procedures				Experimental protocol					
	TMS intensity (% max output)*	VL MEP at 90% MVC (% M_{max})	HS MEP at 90% MVC (% M_{max})	TMS/FNS SIT ratio at 90% MVC in setup†	VL MEP at 100% MVC (%M_{max})	HS MEP at 100% MVC (%M_{max})	FNS SIT at 100% MVC (N)	TMS SIT at 100% MVC (N)	FNS VA (%)	TMS VA (%)
Ineligible										
A16 (M)	50-65	33-42†	10-15	0.23-0.29†	n/a	n/a	n/a	n/a	n/a	n/a
A18 (M)	55-65	7-9†	8-20†	0.16-0.37†	n/a	n/a	n/a	n/a	n/a	n/a
A19 (M)	55-65	27-49	5-8	0.34-0.62†	n/a	n/a	n/a	n/a	n/a	n/a
A20 (F)	50-65	21-31†	10-17	0.45†	n/a	n/a	n/a	n/a	n/a	n/a
A21 (F)	50-55	32†	30‡	0.14†	n/a	n/a	n/a	n/a	n/a	n/a
A22 (M)	55-65	21-24†	3-5	0.43-0.47†	n/a	n/a	n/a	n/a	n/a	n/a
Eligible										
A3 (M)	65	60.4	12.1	0.87	54.9	13.1	8.6	8.5	87.8	88.0
A4 (M)	35	61-87	6-9	0.81	68.8	4.8	7.8	7.4	93.0	93.3
A5 (F)	55	57.3	12.3	1.33	40.0	15.2	7.0	5.3	90.3	92.7
A6 (M)	40	57	11.6	1.46	50.5	23.3	13.3	12.7	86.0	86.6
A7‡ (M)	53	49.3	15	0.95	48.5	24.8	19.8	13.0	75.5	84.0
A8 (M)	40	56.6	3.6	1.21	49.1	9.8	25.1	23.9	71.8	73.2
A9‡ (M)	75	42.2	8.5	1.00	21.4	19.5	7.6	3.4	90.2	95.6
A10‡ (M)	85	41.5	3.9	0.81	38.2	14.6	16.3	13.0	81.3	85.1

F = female; HS = medial hamstrings; FNS = femoral nerve stimulation; M = male; MEP = motor evoked potential; M_{max} = maximal compound muscle action potential; MVC = maximal voluntary contraction; TMS = transcranial magnetic stimulation; SIT = superimposed twitch; VL = vastus lateralis. *For ineligible participants, the TMS intensities in the table are the range of intensities tested in setup procedures. For eligible participants, the TMS intensity in the table was the one in setup procedures that led to the best SIT ratio and was used in subsequent testing. †Part of the exclusion decision for that participant. ‡The modified TMS procedure did not lead to an accurate measure of voluntary activation.

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Table 3. Recommended procedures for use of transcranial magnetic stimulation to assess voluntary activation of the knee extensors in healthy individuals.

Step	Description
1	*HS M_{max}.* Test for presence of HS M-wave using SNS. If HS M_{max} cannot be obtained, discontinue testing. If HS M_{max} is obtained, proceed to Step 2.
2	*VL and VM M_{max}.* Test for presence of VL and VM M-wave using FNS. If VL and VM M_{max} cannot be obtained or participants do not tolerate stimulation, discontinue testing. If VL and VM M_{max} are obtained and participants are tolerant of stimulation, proceed to Step 3.
3	*MVC.* Give participant 2-4 trials to establish MVC. Provide visual feedback. Use the best MVC for subsequent procedures and calculations.
4	*TMS "hot spot."* Start with a low TMS intensity (30-50% output) that evokes MEPs in the knee extensor muscles at rest. If MEPs are not present at rest, then assess MEPs during a weak voluntary contraction of the knee extensors (5-10% MVC). Move the coil in a grid-like manner to identify the "hot spot" – i.e., the coil position that evokes the largest VL and/or VM MEPs. Mark the spot on a cap worn by the participant. If the participant is tolerant of the stimulation, proceed to Step 5.
5	*SIT from FNS at 90% MVC.* Test the size of the SIT at 90% MVC with FNS. This measurement is used in the computation of the SIT ratio in Step 6.
6	*TMS intensity at 90% MVC.* The goal of Step 6 is to identify the TMS intensity that maximizes the size of the SIT at 90% MVC, such that the TMS SIT divided by the FNS SIT is ≥0.80 (i.e., SIT ratio). Using a TMS intensity of ~55% stimulator output, test SITs and peak-to-peak amplitudes of VL, VM, and HS MEPs at 90% MVC. This TMS intensity may or may not maximize the size of the SIT. Thus, test other TMS intensities. Conduct 1-3 trials at each TMS intensity. However, to avoid fatigue, provide adequate rest (2 minutes) between trials and minimize the number of TMS intensities tested and the number of trials at each intensity. Discontinue testing if no TMS intensity leads to a SIT ratio ≥0.80 or if no TMS intensity leads to a VL MEP ≥40% M_{max} (even if the SIT ratio is ≥0.80). If one or more TMS intensities leads to a SIT ratio ≥0.80 and VL MEP ≥40% M_{max}, then, in subsequent testing of voluntary activation, use the intensity that evokes the largest SIT. Use the resting twitch from FNS in the computation of voluntary activation measured by TMS.

HS = medial hamstrings; FNS = femoral nerve stimulation; MEP = motor evoked potential; M_{max} = maximal compound muscle action potential; MVC = maximal voluntary contraction; TMS = transcranial magnetic stimulation; SIT = superimposed twitch; SNS = sciatic nerve branch stimulation; VL = vastus lateralis.

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