Motor cortical and corticospinal function differ during an isometric squat compared with isometric knee extension

Callum G. Brownstein¹ | Paul Ansdell¹ | Jakob Škarabot¹ | Ash Frazer² | Dawson Kidgell² | Glyn Howatson¹,³ | Stuart Goodall¹ | Kevin Thomas¹

¹Faculty of Health and Life Sciences, Department of Sport, Exercise & Rehabilitation, Northumbria University, Newcastle, UK
²Department of Physiotherapy, Faculty of Medicine, Nursing and Health Sciences, School of Primary and Allied Health Care, Monash University, Melbourne, Victoria, Australia
³Water Research Group, School of Environmental Sciences and Development, Northwest University, Potchefstroom, South Africa

Correspondence
Kevin Thomas, Faculty of Health and Life Sciences, Department of Sport, Exercise & Rehabilitation, Northumbria University, Newcastle upon Tyne NE1 8ST, UK. Email: kevin2.thomas@northumbria.ac.uk

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Abstract
It has been suggested that task-specific changes in neurophysiological function (neuroplasticity) should be assessed using testing modalities that replicate the characteristics of the intervention. The squat is a commonly prescribed resistance exercise that has been shown to elicit changes in CNS function. However, previous studies have assessed squat-induced neuroplasticity using isometric knee extension, potentially confounding the results. The aim of the present study was to assess the agreement between corticospinal and intracortical activity relating to the knee extensors during isometric knee extension compared with an isometric squat task. Eleven males completed a neurophysiological assessment in an isometric squat (IS) and knee-extension (KE) task matched for joint angles (hip, knee and ankle). Single- and paired-pulse transcranial magnetic stimulation was delivered during isometric contractions at a range of intensities to assess short-interval cortical inhibition (SICI) and corticospinal excitability. Group mean values for SICI (70 ± 14 versus 63 ± 12% of unconditioned motor evoked potential during IS and KE, respectively) and corticospinal excitability (mean differences 2–5% of the maximal compound muscle action potential at 25, 50, 75 and 100% maximal voluntary contraction between the IS and KE) were not different between the two tasks (P > 0.05) in the vastus lateralis. However, limits of agreement were wide, with poor-to-moderate average intraclass correlation coefficients (ICCs) (SICI, ICC₃,₁ = 0.15; corticospinal excitability, average ICC₃,₁ range = 0.0–0.63), indicating disparate corticospinal and intracortical activity between the IS and KE. These data highlight the importance of task specificity when assessing the modulation of corticospinal excitability and SICI in response to interventions resulting in neuroplastic changes.

Keywords
squat, task specificity, transcranial magnetic stimulation

1 INTRODUCTION

In recent years, there has been an increase in the number of studies applying transcranial magnetic stimulation (TMS) in sport and exercise and movement sciences to assess intracortical and corticospinal activity in response to various interventions (Brownstein et al., 2017; Thomas, Toward, West, Howatson, & Goodall, 2017b; Weier & Kidgell, 2012). Single-pulse TMS permits the quantitative assessment of corticospinal excitability through the size of the compound electromyography (EMG) response, whereas paired-pulse TMS separated by 2–5 and 10–15 ms can be used to examine intracortical inhibitory (termed short-interval intracortical inhibition; SICI) and facilitatory circuits (termed intracortical facilitation; ICF), respectively (Kujirai et al., 1993). Single- and paired-pulse TMS protocols have been used as tools to investigate responses to exercise, such as fatiguing isometric single-limb contractions (Goodall, Howatson, & Thomas, 2018; Hunter, McNeil, Butler, Gandevia, & Taylor, 2016; Kennedy, McNeil, Gandevia, & Taylor, 2016) and locomotor exercise (Brownstein et al., 2017; Sidhu, Cresswell, & Carroll, 2012; Thomas, Dent, Howatson, & Goodall, 2017a), mechanisms of locomotion (Sidhu, Cresswell, & Carroll, 2013b) and neural adaptations to strength training (Weier & Kidgell, 2012).

Many studies have used TMS to assess neural responses to whole-body, dynamic exercise, but a common feature amongst these studies is that responses were assessed in a single-limb, isometric model (Brownstein et al., 2017; Weier & Kidgell, 2012). As such, a discrepancy exists between the neuromechanics of the intervention...
and the testing modality used to detect changes in intracortical and corticospinal activity in response to the interventions. The discrepancy between intervention and testing modality has been highlighted previously by Avela and Gruber (2010), Sidhu, Cresswell, and Carroll (2013a) and, more recently, by Kalmar (2018), who suggested that future studies using TMS to assess neuromuscular responses to whole-body exercise should use testing modalities that more closely replicate the characteristics of the intervention. In support of this supposition, considerable evidence suggests that when assessing neuroplasticity after an intervention, the motor task performed for testing should mirror the motor task(s) performed during the intervention. For instance, Schubert et al. (2008) and Beck et al. (2007) found that intracortical, corticospinal and spinal adaptations to two separate motor training tasks (4 weeks of stability or ballistic training) were constrained to the trained task and were not apparent when performing the non-trained motor task. More recently, Giboin, Weiss, Thomas, and Gruber (2018) compared neuroplasticity responses to two different modalities of isometric strength training (maximal isometric explosive or slow sustained knee extension) and demonstrated that corticospinal adaptations were evident when responses were measured during the trained task, but not for the untrained task. These findings corroborate numerous other studies that have found that plasticity of the CNS is specific to the task trained (Jensen, Marstrand, & Nielsen, 2005; Liepert, Classen, Cohen, & Hallett, 1998; Muellbacher, Ziemann, Boroojerdi, Cohen, & Hallett, 2001). Additionally, postural differences between motor tasks can have large effects on evoked responses (Baudry, Collignon, & Duchateau, 2015; Nuzzo, Trajano, Barry, Gandevia, & Taylor, 2016), adding further support to the notion that testing posture and contraction type should be specific to the trained task.

During a period of strength training, it is well documented that improvements in force production in the first ~4 weeks precede significant structural adaptations (Carroll, Riek, & Carson, 2001; Gabriel, Kamen, & Frost, 2006), indicating that adaptations within the CNS are the primary explanatory factor for improvements in strength. One common training modality for improving lower limb strength is the squat. Previous studies have used the squat in both chronic training (Weier & Kidgell, 2012) and acute bout scenarios (Thomas et al., 2017b), assessing neurophysiological function pre- and post-intervention. Weier and Kidgell (2012) and Weier, Pearce, and Kidgell (2012) both showed alterations in CNS function after 4 weeks of heavy-load squat training, whereas Thomas et al. (2017b) found no changes in corticospinal or intracortical activity when assessing the neuromuscular basis of acute performance enhancement in the minutes after a heavy-resistance squat protocol, despite inducing an increase in jump performance. However, much like the issues highlighted by Sidhu et al. (2013a) and Avela and Gruber (2010), the evoked CNS responses in the aforementioned studies were recorded in single-limb isometric knee extension, rather than the motor task (squat) performed during the intervention. If strength can be mediated by a neuromuscular response to a training stimulus, then the optimal method to assess the alterations in corticospinal and intracortical mechanisms of neuroplasticity might be during the motor task performed throughout the intervention. Thus, it is unclear whether, given the importance of testing specificity, intracortical and corticospinal adaptations in response to squat interventions could be masked if assessments are conducted using testing modalities that are dissimilar to the imposed intervention.

In order to elucidate the appropriateness of using isometric knee extension to assess adaptations to squat exercise, it is first important to identify whether differences exist in intracortical and corticospinal activity between knee-extension exercise and a testing modality that more closely replicates the characteristics of a squat exercise (i.e. a bilateral, multi-joint movement comprising axial loading). The present study aimed to investigate and compare intracortical and corticospinal responses to single- and paired-pulse TMS in the ‘traditional’ isometric knee-extension (KE) set-up, and a joint angle-matched equivalent isometric squat (IS) set-up. Given the differences in the biomechanical characteristics of KE and IS exercise, we hypothesized that there would be limited agreement between corticospinal excitability, SICI and ICF during the two motor tasks.

## 2 METHODS

### 2.1 Ethical approval

The study received ethical approval from the Northumbria University Faculty of Health & Life Sciences Ethics committee (HLSCB251115) in accordance with the ethical standards established in the Declaration of Helsinki, with the exception of registration in a database.

### 2.2 Participants

Eleven young male adults (age, 27 ± 4 years; stature, 181 ± 7 cm; mass, 86.6 ± 15.6 kg) gave written informed consent to take part in the study. Participants were recreationally active, resistance-trained males and reported squatting at least once a week, were free of any cardiorespiratory, neurological or neuromuscular health disorders, had no metal plates in the head/brain and were not taking any medication that might have interfered with the nervous system. All participants...
completed a TMS safety screening questionnaire before the data-collection procedure (Keel, Smith, & Wassermann, 2001). Participants were required to refrain from alcohol consumption and strenuous physical activity in the 24 h before data collection and to abstain from caffeine consumption for 12 h before each experimental visit.

2.3 | Design

Participants visited the laboratory on one occasion and performed a series of submaximal and maximal isometric contractions in two exercise modalities, unilateral isometric knee extension (KE) and bilateral isometric squat (IS), with both conditions matched for hip and knee angle (90 deg) to avoid muscle length–related differences in neural recruitment (Behrens, 2017; Doguet et al., 2017). Participants were familiarized with the study procedures immediately before data collection, including habituation with performing IS and KE exercise and receiving TMS during submaximal contractions. Furthermore, all participants had previously taken part in studies in our laboratory involving measures of TMS recorded in the knee extensors and were thus familiar with performing maximal voluntary contractions (MVCs) and receiving TMS during submaximal contractions. The conditions were pseudorandomized, with a 30 min rest given between the two conditions in order to minimize the influence of fatigue. During both conditions, participants received single- and paired-pulse TMS and electrical stimulation of the femoral nerve whilst performing submaximal and maximal isometric contractions. Corticospinal excitability, SICI and ICF, the maximal compound muscle action potential \( M_{\text{max}} \) and the EMG–force relationship were measured in the vastus lateralis (VL) and rectus femoris (RF) using surface EMG. These variables were then compared between the two conditions.

2.4 | Procedures

2.4.1 | Isometric knee extension

A calibrated load cell (MuscleLab force sensor 300; Ergotest technology, Prosgrunn, Norway) was used to measure isometric knee extensor force (in newtons). The load cell was fixed to a custom-built chair and strapped with a non-compliant cuff to the participant’s right leg, superior to the ankle malleoli. During contractions, participants were instructed to grasp the handles on the side of the chair for support during MVCs. Participants were instructed to maintain hip and knee angles at 90 deg flexion during contractions, with these joint angles measured using a goniometer at the beginning of the trial and visually inspected by the investigators during contractions to ensure that no change in joint angle occurred. Three MVCs were performed before the trial, with 60 s between each contraction. In order to control for any learning effect on performing MVCs during IS exercise, participants were asked to perform an additional MVC if there was a >5% increase in force during successive MVCs. This was performed until three consecutive MVCs were performed with force values within 5% of each other. Six participants were required to perform one additional MVC. The maximal force from the MVCs was recorded in order to calculate the submaximal contraction values. The force trace was displayed on a computer screen directly in front of participants in order to assist in providing maximal efforts during MVCs and to provide the target force during submaximal contractions. Target forces were set using guidelines and real-time force feedback (Spike2; CED, Cambridge, UK).

2.4.2 | Isometric squat

Isometric squat force (in newtons) was measured using a force plate placed directly under the right foot (type 9286B; Kistler Group, Winterthur, Switzerland). In order to provide support during isometric contractions, participants were seated on a bench directly under a fixed barbell, with knee and hip angle maintained at 90 deg flexion measured using a goniometer (Figure 1). This procedure was implemented after pilot testing revealed that when participants were unsupported, rather than being seated on a bench, the contraction intensity and level of EMG activity required to support their own body weight whilst maintaining knee and hip angle at 90 deg flexion was too high to allow the measurement of SICI (Ortu, Deriu, Suppa, Tolu, & Rothwell, 2008). The barbell height was adjusted at the beginning of each trial based on the torso length of the participant and was positioned on the shoulders (high-bar position). The participants’ feet were positioned hip width apart with toes pointing forwards, with foot position determined at the beginning of the trial and marked to ensure consistent placement throughout the trial. Participants held the barbell during contractions and were given freedom to choose their hand position, which was maintained throughout the trial. During contractions, participants were instructed to exert force upwards against the bar using their whole body (Bishop et al., 2017). The investigators visually inspected hip and knee angle during contractions to ensure that no change in joint angle occurred. Three MVCs were performed before the trial, with 60 s between each contraction. In order to control for any learning effect on performing MVCs during IS exercise, participants were asked to perform an additional MVC if there was a >5% increase in force during successive MVCs. This was performed until three consecutive MVCs were performed with force values within 5% of each other. Six participants were required to perform one additional MVC. The maximal force from the MVCs was recorded in order to calculate the submaximal contraction values. The force trace was displayed on a computer screen directly in front of participants in order to assist in providing maximal efforts during MVCs and to provide the target force during submaximal contractions.

2.4.3 | Isometric contraction protocol

During assessment of corticospinal excitability in KE and IS trials, seven sets of brief \( (\sim 3 \text{ s}) \) isometric contractions were performed at 25, 50, 75 and 100% MVC. Contraction intensities were randomized, and participants were given 60 s rest between each contraction and 3 min between each set to avoid the potential influence of fatigue on motor evoked potential (MEP) properties. Two electrical nerve stimuli and five TMS pulses were delivered at each contraction intensity. For assessment of SICI and ICF, 40 stimuli (20 single pulses and 20 paired pulses) were delivered in six sets of six and one set of four
during a 10% MVC, with 30 s between each set (see Instrumentation for details).

2.5 | Instrumentation

2.5.1 | Electromyography recordings

The EMG activity was recorded from RF, VL and biceps femoris (BF), with a reference electrode placed on the patella; the areas underneath were cleaned and shaved before electrode placement. Surface electrodes (Ag/AgCl; Kendall H87PG/F, Covidien, Mansfield, MA, USA) were placed 2 cm apart over the muscle belly. The electrodes recorded electrical activity in the VL, RF and BF, with the signal processed to permit analysis of the root mean square (RMS) amplitude for voluntary contractions, the compound muscle action potential (M-wave) elicited by electrical stimulation of the femoral nerve, and the MEP elicited by TMS. Signals were amplified, with gain $\times 1000$ for EMG and $\times 300$ for KE force (CED 1902; Cambridge Electronic Design, Cambridge, UK), bandpass filtered (EMG only; 20–2000 Hz), digitized (4 kHz; CED 1401; Cambridge Electronic Design) and analysed offline. Further details on these methods are provided in the Percutaneous nerve stimulation and Transcranial magnetic stimulation sections below.

2.5.2 | Percutaneous nerve stimulation

Percutaneous stimulation of the right femoral nerve was administered using square-wave pulses (200 $\mu$s) via a constant-current stimulator (DS7AH; Digitimer Ltd, Welwyn Garden City, UK) using self-adhesive
surface electrodes (CF3200; Nidd Valley Medical Ltd, Harrogate, UK). The cathode was placed over the femoral nerve high in the femoral triangle, and the anode between the greater trochanter and iliac crest. Cathode placement was adjusted to elicit the greatest $M_{\text{max}}$ amplitude in the VL. Stimulations were delivered in 20 mA stepwise increments beginning at 20 mA until the maximal quadriceps twitch amplitude ($Q_{\text{tw}}$, in newtons) and muscle compound action potential ($M_{\text{max}}$, in millivolts) in the VL were elicited. The resulting intensity was then increased by 30% in order to ensure that the stimulation intensity was supramaximal. This procedure was conducted during both KE and IS exercise to ensure that the stimulation intensity was supramaximal under both modalities, with stimulation intensities of 229 ± 119 mA during KE and 260 ± 100 mA during IS.

### 2.5.3 Transcranial magnetic stimulation

Single- and paired-pulse TMS was delivered over the motor cortex via a concave double-cone coil using a BiStim unit and two Magstim 200$^2$ stimulators (The Magstim Company Ltd, Whitland, UK). The junction of the double-cone coil was aligned tangentially to the sagittal plane, with its centre 1–2 cm to the left of the vertex. The optimal coil placement was determined at the start of each trial as the position that elicited the largest MEP in the VL muscle at 50% stimulator output during a 10% MVC. This procedure was conducted separately during both KE and IS exercise to ensure optimal coil placement during both modalities. The position was then marked with indelible ink to ensure consistent placement throughout the trial. The stimulator intensity was based on active motor threshold (AMT) during a 10% MVC during each condition. The AMT was defined as the intensity that elicited a MEP amplitude of >200 μV in three out of five stimulations in the VL (Weier & Kidgell, 2012). We believed it was more appropriate to base AMT and stimulator output on responses in the VL rather than the RF, which has a bi-articular make-up and is involved in both hip and knee extension, potentially influencing the level of recruitment during the IS and KE and thereby confounding intracortical and corticospinal responses. For single-pulse TMS, the stimulus intensity was set at 120% AMT. The configuration used during paired-pulse TMS consisted of a conditioning stimulus intensity of 70% AMT with an interstimulus interval (ISI) of 2 ms for SICI, and a conditioning stimulus intensity of 60% AMT with an ISI of 10 ms for ICF. The suprathreshold test pulse intensity was maintained at 120% AMT for both SICI and ICF. Pilot work from our laboratory has identified that this configuration elicits the highest degree of SICI and ICF in the active knee extensors, while 20 single-pulse and 20 paired-pulse TMS stimuli were identified as the minimal number required to obtain an accurate estimate of SICI and ICF.

### 2.6 Data analysis

The peak-to-peak amplitudes of the EMG responses to motor nerve stimuli and TMS were analysed offline. The root mean square EMG amplitude ($\text{RMS}_{\text{EMG}}$) and average force were calculated in the 500 ms before each TMS stimulus to ensure a similar level of background muscle activity during each stimulation when assessing SICI and ICF and to assess the EMG–force relationship at different contraction intensities. For the latter, $\text{RMS}_{\text{EMG}}$ at a given contraction intensity was normalized to $\text{RMS}_{\text{EMG}}$ during the mode-specific 100% MVC. To quantify SICI and ICF, the ratio of the average conditioned paired-pulse MEP amplitude was expressed relative to the average unconditioned MEP amplitude at 120% AMT. A conditioned versus unconditioned ratio <100% indicates inhibition, whereas a ratio >100% indicates facilitation. If the ratio for SICI was >100% or the ratio for ICF was <100%, the data from the corresponding participant were removed from the analysis. In order to assess corticospinal excitability and EMG activity at different contraction intensities, the MEP amplitude and $\text{RMS}_{\text{EMG}}$ were averaged across the five TMS pulses and normalized to the $M_{\text{max}}$ assessed at each contraction intensity ($\text{MEP/M}_{\text{max}}$ and $\text{RMS/M}_{\text{max}}$, respectively).

### 2.7 Statistical analyses

SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. All data are presented as means ± SD unless stated otherwise. Significance was set at an $\alpha$ level of 0.05. Normality of data was assessed using the Shapiro–Wilk test. If the data were not normal, transformations were performed using common logarithm or square root. In order to demonstrate that there were no learning or fatigue effects associated with performing multiple MVCs during IS and KE exercise, a repeated-measures ANOVA was performed to assess for differences in MVC values obtained throughout the contraction protocol. Analysis revealed no significant main effect of time on MVC scores in either KE ($F_{6,60} = 2.736, P = 0.084$, partial eta squared ($\eta^2_p$) = 0.215) or IS ($F_{6,60} = 2.539, P = 0.086$, $\eta^2_p$ = 0.215). Furthermore, reliability analysis revealed excellent reliability for both IS (intraclass correlation coefficients (ICCs) ICC$_{3,1}$ = 0.96, 95% confidence interval (CI) 0.90–0.99) and KE MVCs (ICC$_{3,1}$ = 0.96, 95% CI 0.90–0.99), with low coefficients of variation for both modalities (2.9 and 1.9% for IS and KE, respectively). Student's paired $t$ test was performed to determine the difference in SICI between the IS and KE. Agreement between modalities was assessed graphically using Bland–Altman plots, with lines indicating the mean difference and the 95% limits of agreement (mean difference ± 2SD of the difference). Absolute agreement between contraction modalities was assessed using two-way mixed-effect ICCs (ICC$_{3,1}$) with 95% CIs. According to the guidelines recommended by Koo and Li (2016), ICCs between 0.5 and 0.75 were considered as moderate agreement, values between 0.75 and 0.9 as good agreement, and values >0.9 considered as excellent agreement. Sphericity was assessed using Mauchly's test. In the event of a violation, the Greenhouse–Geisser correction was used. A $2 \times 2$ repeated-measures ANOVA [two types of stimuli (single and paired pulse) and two modalities (IS and KE)] was used to assess differences in RMS during investigation of SICI. A $4 \times 2$ repeated-measures ANOVA [four contraction intensities (25, 50, 75 and 100%) and two modalities (IS and KE)] was used to assess differences between the modalities in $M_{\text{max}}$, $\text{RMS/M}_{\text{max}}$, $\text{MEP/M}_{\text{max}}$ and BF EMG during different contraction intensities. In the event of significant main effects, analysis was continued using pairwise comparisons with Bonferroni correction. The $\eta^2_p$ was reported as a measure of effect size. An interaction was reported only when it was found to be statistically significant.
FIGURE 2  Short-interval intracortical inhibition (SICI) during the isometric squat (IS) and knee extension (KE) measured in the rectus femoris and vastus lateralis, with values displayed at the group level (a) as the mean + SD (filled bars, IS; open bars, KE), as individual data points (b) during the IS relative to KE (filled circles, vastus lateralis; open circles, rectus femoris), with the dashed line representing the line of agreement ($n = 11$), and as Bland–Altman plots (c,d), with systemic bias (continuous lines) and 95% limits of agreement (dashed lines) showing agreement between the modalities. Abbreviation: MEP, motor evoked potential

The EMG–force relationship was estimated by linear regression, with the determination coefficient considered acceptable at $r^2 > 0.95$ and $P < 0.05$.

3 | RESULTS

3.1 | Short-interval intracortical inhibition and facilitation

We were unable to induce SICI during the IS trial for one subject (conditioned versus unconditioned ratio >100%); as such, this participant was excluded from further analysis. On average, SICI was similar in the IS compared with KE trial in both VL (70 ± 14 versus 63 ± 12%; $t_9 = 1.330, P = 0.216$) and RF (58 ± 19 versus 71 ± 19%; $t_9 = −1.577, P = 0.149$; Figure 2a). No difference was found in pre-stimulation EMG activity during KE and IS at 10% MVC between single- and paired-pulse stimulation in the RF (IS, 0.030 ± 0.005 versus 0.030 ± 0.005 mV; KE, 0.040 ± 0.014 versus 0.040 ± 0.011 mV; $F_{1,9} = 0.322, P = 0.584, \eta_p^2 = 0.035$) or VL (IS, 0.044 ± 0.016 versus 0.045 ± 0.016 mV; KE, 0.081 ± 0.128 versus 0.081 ± 0.128 mV; $F_{1,9} = 0.606, P = 0.456, \eta_p^2 = 0.063$). Despite this, the agreement between modalities was poor to moderate; ICC$_{3,1}$ for SICI was 0.15 (95% CI 0.00–0.67) in the VL and 0.09 (95% CI 0.00–0.63) in the RF (Figure 2b). Limits of agreement were −25 to 39% and −62 to 37%; systemic bias, 7 and −13%, in the VL and RF, respectively (Figure 2c,d).

We were able to induce ICF during both the IS and the KE trials in only five and two subjects in RF and VL, respectively (conditioned versus unconditioned ratio >100%). In all other subjects, we were unable to elicit ICF in either modality, with conditioned versus unconditioned ratios <100%. Owing to the small number of valid cases, no statistical analyses were performed for ICF.

3.2 | Maximal compound action potential

On average, $M_{\text{max}}$ was similar in KE and the IS in both VL (5.5 ± 1.8 versus 5.0 ± 1.5 mV; $F_{1,10} = 2.106, P = 0.177, \eta_p^2 = 0.174$) and RF (5.8 ± 2.2 versus 5.9 ± 2.6 mV; $F_{1,10} = 0.013, P = 0.911, \eta_p^2 = 0.001$). However, agreement between modalities varied at different contraction intensities, with ICC$_{3,1}$ values ranging between poor-to-moderate and moderate-to-excellent (Table 1). The $M_{\text{max}}$ was also similar across different contraction intensities in VL ($F_{1.4,14.3} = 1.106, P = 0.337, \eta_p^2 = 0.100$) and RF ($F_{2.1,21.1} = 0.907, P = 0.424, \eta_p^2 = 0.083$).
testing showed that RMS/Mmax was greater during KE compared with IS at 50% MVC (4 ± 1 versus 2 ± 1%; P = 0.013).

In RF, RMS/Mmax was higher on average during KE compared with the IS (F1,10 = 10.688, P = 0.008, ηp² = 0.517; Figure 4a). Furthermore, agreement for RMS/Mmax between the IS and KE in RF ranged from poor to poor-to-moderate at different contraction intensities (Table 1; Figure 4b). Both modalities were also modulated by contraction intensity (F3,30 = 174.329, P < 0.001, ηp² = 0.946) such that RMS/Mmax increased with greater contraction intensity (P < 0.005; Figure 4a).

The determination coefficient of linear regression was significant in both the VL and RF for both modalities, suggesting that the force–EMG relationship was linear in all cases (see Figure 5). On average, the antagonist EMG activity was similar between KE and IS (0.06 ± 0.05 versus 0.05 ± 0.02 mV; F1,10 = 1.722, P = 0.219, ηp² = 0.147), but it was affected by contraction intensity (F1,12,12 = 24.179, P < 0.001, ηp² = 0.707) insofar as BF EMG activity increased stepwise from 25 to 100% MVC (KE, 0.04 ± 0.01, 0.05 ± 0.02, 0.08 ± 0.06 and 0.09 ± 0.08 mV; and IS, 0.04 ± 0.01, 0.04 ± 0.01, 0.05 ± 0.01 and 0.06 ± 0.02 mV; P < 0.05).

4 | DISCUSSION

The aim of the present study was to compare corticospinal and intracortical responses to single- and paired-pulse TMS during an IS and KE exercise. The key finding from the study was that the two motor tasks resulted in disparate corticospinal and intracortical activity, with a poor level of agreement between the two exercise modalities. Specifically, despite a similar level of background EMG during measurements of SICI and a comparable response at a group level, the absolute agreement assessed through ICCs in the VL and RF was poor-to-moderate, and limits of agreement were wide, indicating disparate activity of inhibitory interneurons during the tasks. Likewise, comparable responses were observed at a group level between normalized MEP amplitude in response to single-pulse TMS delivered at a range of contraction intensities in the VL and RF, but agreement between the tasks was generally poor, with ICCs ranging from poor to poor-to-good, and wide limits of agreement at most contraction intensities. Collectively, these results highlight the task-specific nature of corticospinal and intracortical activity and could have implications regarding the requirement for testing specificity when assessing CNS responses.

3.3 | Motor evoked potentials

On average, MEP/Mmax was similar between IS and KE in both VL (F1,10 = 1.062, P = 0.327, ηp² = 0.096) and RF (F1,10 = 2.407, P = 0.152, ηp² = 0.194; Figure 3a). However, the agreement between the modalities varied between MEP/Mmax measured at different contraction intensities, with average ICCs3,1 values >0.6 and ≤0.63 (Table 1; Figure 3b). Bland–Altman plots with limits of agreement and systematic bias are displayed in Figures 3c–f. In both modalities, MEP/Mmax was modulated by contraction intensity in both VL (F3,30 = 14.826, P < 0.001, ηp² = 0.597) and RF (F3,30 = 11.153, P < 0.001, ηp² = 0.527; Figure 3a) such that MEP/Mmax was smaller at 25% MVC compared with higher contraction strength in both muscles (P ≤ 0.025). In RF, there was also a statistically significant modality × contraction intensity interaction for MEP/Mmax (F3,30 = 3.267, P = 0.035, ηp² = 0.246). Specifically, the post hoc test revealed that MEP/Mmax was smaller during IS compared with KE at 25% MVC (24 ± 23 versus 47 ± 20%; P = 0.004).

3.4 | Electromyography and force–EMG relationship

In VL, RMS/Mmax was similar in both modalities on average (F1,10 = 2.695, P = 0.132, ηp² = 0.212; Figure 4a), but the agreement between them was generally poor, with ICCs3,1 values ranging from poor to moderate-to-good (Table 1; Figure 4b). However, the RMS/Mmax in VL was influenced by the contraction intensity (F3,30 = 111.389, P < 0.001, ηp² = 0.918), in that it was greater with increased contraction strength (P < 0.005; Figure 4a). There was also statistically significant modality × contraction intensity interaction for RMS/Mmax in VL (F1,4,14,1 = 10.242, P = 0.004, ηp² = 0.506). Post hoc

### Table 1

Intraclass correlation coefficients (ICC3,1; with 95% confidence intervals) displaying the level of agreement between the isometric squat and knee extension in the vastus lateralis and rectus femoris during different contraction intensities for Mmax, MEP/Mmax and RMS/Mmax (n = 11).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Contraction intensity</th>
<th>Vastus lateralis</th>
<th>Rectus femoris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mmax</td>
<td>25% MVC</td>
<td>0.92 (0.72–0.98)</td>
<td>0.33 (0.00–0.77)</td>
</tr>
<tr>
<td></td>
<td>50% MVC</td>
<td>0.64 (0.08–0.89)</td>
<td>0.08 (0.00–0.55)</td>
</tr>
<tr>
<td></td>
<td>75% MVC</td>
<td>0.36 (0.00–0.77)</td>
<td>0.33 (0.00–0.77)</td>
</tr>
<tr>
<td></td>
<td>100% MVC</td>
<td>0.42 (0.00–0.79)</td>
<td>0.25 (0.00–0.73)</td>
</tr>
<tr>
<td>MEP/Mmax</td>
<td>25% MVC</td>
<td>0.15 (0.00–0.68)</td>
<td>0.34 (0.00–0.74)</td>
</tr>
<tr>
<td></td>
<td>50% MVC</td>
<td>0.59 (0.00–0.87)</td>
<td>0.32 (0.00–0.76)</td>
</tr>
<tr>
<td></td>
<td>75% MVC</td>
<td>0.63 (0.08–0.88)</td>
<td>0.22 (0.00–0.45)</td>
</tr>
<tr>
<td></td>
<td>100% MVC</td>
<td>0.41 (0.00–0.79)</td>
<td>0.00 (0.00–0.24)</td>
</tr>
<tr>
<td>RMS/Mmax</td>
<td>25% MVC</td>
<td>0.54 (0.00–0.85)</td>
<td>0.12 (0.00–0.52)</td>
</tr>
<tr>
<td></td>
<td>50% MVC</td>
<td>0.00 (0.00–0.35)</td>
<td>0.01 (0.00–0.45)</td>
</tr>
<tr>
<td></td>
<td>75% MVC</td>
<td>0.31 (0.00–0.76)</td>
<td>0.07 (0.00–0.53)</td>
</tr>
<tr>
<td></td>
<td>100% MVC</td>
<td>0.41 (0.00–0.80)</td>
<td>0.00 (0.00–0.44)</td>
</tr>
</tbody>
</table>

Abbreviations: MEP, motor evoked potential; Mmax, maximal compound muscle action potential; MVC, maximal voluntary contraction; and RMS, root mean square.
SICI when measured in the knee extensors, suggesting that the poor agreement between the modalities is not simply a result of variability in the measures (O’Leary, Morris, Collett, & Howells, 2015). Furthermore, the limits of agreement for SICI were ±22 and ±50% in the VL and RF, respectively. These limits of agreement are wide in the context of previously observed changes in SICI measured in the knee extensors as a consequence of strength training. For example, studies have reported statistically significant changes in SICI ranging between 22 and 35% in response to strength-training interventions (Weier & Kidgell, 2012; Weier et al., 2012). Given that the ICCs for SICI measured during KE and IS in the present study were lower than has previously been reported during isometric knee extension.
**FIGURE 4** Root mean square (RMS) EMG activity at different contraction intensities normalized to the maximal compound action potential (M\(_{\text{max}}\)) at the same contraction intensity in vastus lateralis and rectus femoris during the isometric squat (IS) and knee extension (KE) at different contraction intensities expressed as a percentage of the maximal voluntary contraction (MVC; \(n = 11\)). Values are displayed at the group level (a) as means ± SD, and as individual data points during the IS relative to KE (b), with the dashed line representing the line of agreement, and as Bland–Altman plots, with systemic bias (continuous line) and 95% limits of agreement (dashed lines) at 25 (c), 50 (d), 75 (e) and 100% MVC (f). *P < 0.005 compared with higher contraction intensities. #P < 0.015 compared with the other modality (O’Leary et al., 2015) and that limits of agreement were wider than the magnitude of previously observed changes in SICI in response to strength-training interventions (Weier & Kidgell, 2012; Weier et al., 2012), this implies that the agreement between the two modalities was poor, indicating differences in the activity of intracortical inhibitory interneurons during the tasks.

Although voluntary contraction strength has been shown to influence the degree of SICI (Ortu et al., 2008; Ridding, Taylor,
Rothwell, 1995), the similar relative contraction intensity and background prestimulation EMG in the VL and RF during measurements of SICI in the present study suggest that the disparity between SICI measured in the two modalities was not attributable to differences in the level of motor drive to the muscle. Instead, it is plausible that the differences in the neuromechanics of the IS and KE could have contributed to the lack of agreement between SICI measured in the two conditions. Specifically, the bilateral versus unilateral nature of the IS and KE trials, respectively, could have influenced the level of SICI in the VL and RF. Indeed, it has been reported previously that there are differences in voluntary control of unilateral versus bilateral contractions that could be mediated through alterations in intracortical inhibition (Ferbert et al., 1992; Skarabot, Cronin, Strojnik, & Avela, 2016). For example, during bilateral contractions, it has been suggested that inhibition is modulated through interhemispheric interactions between homologous muscle representations of the primary motor cortex acting to produce a coordinated movement of the two limbs (Oda & Moritani, 1995). Another integral difference between the two conditions that might have contributed to the lack of agreement in SICI is that the KE is a single-joint exercise, in which the quadriceps femoris muscle group is the sole contributor to force production, whereas the IS is a multi-joint exercise, in which additional agonist and synergist muscle groups, including the hip extensors, are activated. It has been speculated that SICI could be involved in the ‘fractionation’ of muscular activity, such that inhibitory influences are reduced on the contracting muscle whilst maintaining or increasing inhibition in the non-contracting muscles (Ortu et al., 2008; Zoghi, Pearce, & Nordstrom, 2003). Although there were no differences in SICI on a group level, the concurrent activation of agonist and synergist muscle groups during the IS could influence the degree of inhibition measured in the knee extensors. In support of this supposition, previous studies have found that concurrent activation of synergist muscles influences the magnitude of SICI measured in a target muscle (Devanne et al., 2002; Kouchtir-Devanne, Capaday, Cassim, Derambure, & Devanne, 2012), possibly owing to interactions between muscle representations within the motor cortex (Capaday, Ether, Van Vreeswijk, & Darling, 2013). Thus, differences in the neuromechanics of muscle recruitment between the IS and KE could have contributed to the lack of agreement in SICI between the two motor tasks.

Similar to measures of SICI, ICCs showed generally poor agreement between corticospinal excitability measured during the KE and IS trials at a range of contraction intensities. Furthermore, the limits of agreement between corticospinal excitability measured during KE and IS ranged from ±42 to ±53% in the RF and from ±28 to ±44% in the VL across different contraction intensities. These limits of agreement are wider than many of the previously reported changes in corticospinal excitability measured in the knee extensors in response to locomotor exercise. For example, Thomas et al. (2017a) reported a statistically significant 5% decrease in corticospinal excitability 24 h after competitive soccer match-play. Likewise, both Goodall et al. (2018) and Jubeau et al. (2014) reported a ~15% increase in corticospinal excitability in response to fatiguing isometric and locomotor exercise, respectively. Given that responses were normalized to the maximum these differences between the tasks could not have been related to differences in neuromyoskeletal transmission at the sarcolemma. It should be noted that at certain contraction intensities, there were differences in the EMG activity in the VL and/or RF muscles between the two modalities. In particular, the EMG in the RF was higher than in the VL across different contraction intensities. These limits of agreement are wider than many of the previously reported changes in corticospinal excitability. This can probably be explained by the plateau in MEP amplitude observed at >50% MVC, which has previously been observed in work conducted in the knee-extensor musculature (Goodall, Howatson, Romer, & Ross, 2009; Sidhu, Bentley, & Carroll, 2009). This observation is probably attributable to a decline in motoneuron output in response to the stimulus, arising from an inability of some motoneurons to fire in response to excitatory input (Goodall, Howatson, Romer, & Ross, 2014; Todd, Taylor, & Gandevia, 2003). Nevertheless, it is possible that the differences in the level of muscle activity could have contributed to the lack of agreement between corticospinal excitability measured during the two modalities.

During multi-joint muscle contractions, the motor cortex and corticospinal tract work as a dynamic and integrated neural network in order to execute the required movement (Capaday et al., 2013; Devanne et al., 2002; Mason et al., 2017). Rather than each muscle group involved in the movement being controlled singly and separately
by distinct territories within the motor cortex, cortical points are interconnected by intrinsic collaterals that function to control muscle synergies in an integrated manner (Capaday et al., 2013). For example, cortical mapping experiments examining the topography of muscle representations within the motor cortex have shown that the areal representations of task-related proximal and distal muscles of the upper limbs overlap considerably, despite differences in the location of their optimal points (Devanne et al., 2006). In the case of the IS, the quadriceps femoris muscles act as the primary agonist muscle group during contraction but are subserved by other agonist and synergist muscles, such as the hip extensors. Given the overlapping and intertwined nature of muscle representations in the motor cortex and corticospinal tract, it is plausible that the activation of synergist muscles during the IS could have contributed to the lack of agreement between corticospinal excitability measured in the KE and IS. In support of this, Devanne et al. (2002) reported differences in corticospinal excitability during a finger-pointing task involving coactivation of multiple muscle groups in the upper limb compared with an isolated contraction of each muscle and suggested that interactions between muscle representations within the motor cortex were responsible for the differential modulation of corticospinal excitability.

Although interactions between muscle representations within the motor cortex could have contributed to the divergence in corticospinal excitability between the two tasks, given that MEP amplitude depends on the level of excitation of the motor cortex and spinal motor neurons, the possibility that there might have been a contribution at the spinal level cannot be ruled out. For example, differences in ‘recruitment gain’ of the motoneuron pool, whereby the range of thresholds for different motoneurons within the pool can be compressed or expanded depending on the nature of the motor task (Kernell & Hultborn, 1990; Vestergaard & Berg, 2015), could have influenced corticospinal excitability measured in the knee extensors. Further insight into the potential spinal contribution during the two motor tasks could be gained from stimulation at the cervicomedullary junction (Taylor & Gandevia, 2004). Although a contribution of spinal factors cannot be ruled out, the lack of agreement in SICI during the motor tasks suggests that intracortical mechanisms contributed, at least in part, to the results of the present study.

In addition to SICI, in the present study we also attempted to measure and compare ICF during the KE and IS trials. In an attempt to induce the maximal level of facilitation, we implemented paired-pulse stimulus variables (ISI and conditioning stimulus intensity), which have previously been optimized during pilot work in our laboratory when assessing ICF in the rectus femoris. Despite this, we were able to induce facilitation during both the IS and KE in only a limited number of participants and, consequently, were unable to make a valid comparison between the two modalities. In particular, we were unable to induce ICF in the VL in most participants during the IS or KE trials, despite AMT being based on responses in the VL. Previous work has likewise shown that ICF demonstrates significant intersubject variability in the knee extensors, such that some individuals do not exhibit facilitation using paired-pulse protocols previously shown to elicit ICF (Kujirai et al., 1993; O’Leary et al., 2015). For example, when attempting to assess the reliability of ICF in the active vastus lateralis, O’Leary et al. (2015) found an average ratio of conditioned/unconditioned MEP amplitude <1.0 in a cohort of 16 participants. It has been suggested that ICF reflects the excitability of glutamate-mediated NMDA excitatory interneurons (Liepert, Schwenkreis, Tegenthoff, & Malin, 1997; Nakamura, Kitagawa, Kawaguchi, & Tsuji, 1997), but this still remains unclear (Ni & Chen, 2011). These results call into question the validity and applicability of measuring ICF in the vastus lateralis.

4.2 Limitations

The present study opens up an interesting area for future research concerning CNS adaptations to squat-based exercise, but it is important to acknowledge the limitations of the study. Although the set-up used during the IS exercise was designed to replicate closely the characteristics of the squat exercise, there are a number of differences between the IS set-up used in the present study compared with a conventional dynamic squat, such as the contraction mode, being supported versus unsupported, and potential differences in joint angles. Nevertheless, our aim was to use a testing modality that closely replicates the characteristics of the squat exercise whilst also allowing us to compare responses with the conventional method used to assess neuroplasticity in response to squat interventions, i.e. isometric knee extension with hip and knee angles of 90 deg (Weier & Kidgell, 2012; Weier et al., 2012). Using an experimental set-up that precisely replicated that of normal squat exercise, i.e. dynamic, unsupported movement under load with self-selected hip and knee angles, would have had obvious methodological impracticalities that would have precluded us from taking neurophysiological measurements in such conditions. However, given the closer biomechanical similarities between the IS and normal squat exercise compared with that of KE, the IS set-up used in the present study has the potential to provide a more valid means of assessing neuroplasticity in response to squat-based interventions, providing an intriguing avenue for future investigations.

4.3 Conclusion

In the present study, we found disparate corticospinal and intracortical responses to single- and paired-pulse TMS in the vastus lateralis and rectus femoris during joint-angle-specific isometric squat and knee-extension exercise, despite similar levels of background EMG during the two modalities. The lack of agreement noted between corticospinal excitability and SICI could have been a consequence of the differences in the characteristics of the tasks, such as the bilateral, multi-joint contraction implicated during the isometric squat compared with the unilateral, single-joint contraction involved during isometric knee extension. The results highlight the task-specific nature of corticospinal and intracortical activity and emphasize the requirement for testing specificity when assessing CNS responses. Future studies should assess differences in the sensitivity of the IS compared with isometric KE in detecting changes in CNS function in response to interventions involving the squat.
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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

Experiments were performed in the Integrated Physiology Laboratory at Northumbria University. C.G.B., P.A., J.S., S.G. and K.T. designed the study protocol. C.G.B., P.A. and J.S. acquired the data. All authors analysed and interpreted the data and drafted or revised the final manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

ORCID

Jakob Škarabot http://orcid.org/0000-0002-0118-1216
Dawson Kidgell http://orcid.org/0000-0002-2271-1298
Glyn Howatson http://orcid.org/0000-0001-8494-2043
Kevin Thomas http://orcid.org/0000-0003-2973-5337

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