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RESEARCH ARTICLE

Control of Movement

Hypertonic saline-evoked muscle pain in the quadriceps reduces neuromuscular performance and alters corticospinal excitability

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Abstract

Muscle pain can alter corticospinal function, but the specific excitatory/inhibitory effects on the quadriceps across different levels of corticospinal neuron recruitment remain unclear. Furthermore, maximal force production is reduced with muscle pain, but how the rate of force development, a key component of neuromuscular function, remains less known. To investigate this, healthy participants completed an isometric maximal voluntary contraction (MVC) followed by submaximal, intermittent contractions after receiving a hypertonic saline injection into the vastus lateralis to cause quadriceps pain (HYP), or isotonic saline, a nonpainful control (ISO). Peripheral nerve stimulation was delivered during and after MVCs to determine neuromuscular function. Transcranial magnetic stimulation (TMS) was delivered at 120% and 150% of active motor threshold during submaximal contractions to determine corticospinal excitability/inhibition, along with paired-pulse TMS to determine short-interval intracortical inhibition (SICI). Results revealed a moderate effect size (ES) reduction in MVC force (ES = −0.68, $P = 0.020$), early-phase rate of force development (ES = −0.57, $P = 0.029$), and voluntary activation (ES = −0.66, $P = 0.008$) in HYP compared with ISO. Corticospinal excitability increased in HYP compared with ISO (ES = 0.60, $P = 0.023$), whereas corticospinal inhibition decreased in HYP at higher stimulation intensities only (ES = 0.63, $P = 0.017$). Conversely, SICI increased in HYP compared with ISO (ES = 0.58, $P = 0.035$). Our findings indicate that muscle pain induced by a hypertonic saline injection reduced quadriceps neuromuscular function due to centrally mediated mechanisms, potentially involving both excitatory and inhibitory effects on the corticospinal tract.

NEW & NOTEWORTHY Hypertonic saline-induced quadriceps muscle pain reduced knee-extensor maximal voluntary force, rate of force development, and voluntary activation, without altering peripheral muscle function, suggesting a centrally mediated impairment of neuromuscular performance in healthy individuals. Alongside these changes was an increase in corticospinal excitability at both low and high stimulation intensities, whereas pain decreased corticospinal inhibition at high stimulation intensities only. Furthermore, hypertonic saline-induced pain increased intracortical inhibition, suggesting nonuniform effects of pain on the corticospinal tract.

afferent feedback; central fatigue; maximal strength; nociception; TMS

INTRODUCTION

Muscle pain is a salient and widespread experience in health and disease. Pain can be defined as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (1). Clinically, individuals with conditions such as fibromyalgia and complex regional pain syndrome can display elevated levels of appendicular muscle pain (1–3). These conditions are also associated with impaired neuromuscular function and reduced voluntary activation, as highlighted in recent meta-analyses (4, 5). The manifestation of muscle pain is also a common experience, both during exercise (exercise-induced pain) (6) or in the days following intense or unaccustomed exercise in the form of mechanical hyperalgesia, commonly referred to as delayed

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onset muscle soreness (7). These pain scenarios typically arise from stimulation of group III/IV nociceptive afferents by noxious concentrations of biochemicals associated with anaerobic energy contribution and inflammation (e.g., hydrogen ions, adenosine, potassium) (8–10). Nociceptive signals transmitted from group III/IV afferents synapse onto the dorsal horn of the spinal cord, where they ascend to several brain areas, including the primary somatosensory cortex, resulting in the perception of pain (6).

In addition to generating a conscious pain perception, nociceptive signals can also impact the function of the primary motor cortex and the corticospinal pathway (11). This is of particular interest because motor performance of the lower limb (primarily the quadriceps femoris) relies on the corticospinal pathway, as it controls voluntary movement, coordinates muscle activation, integrates sensory feedback, and adapts to pain or fatigue (11). Altered function may have negative implications for both exercise performance success and completing activities of daily living (e.g., stair climbing).

A consistently observed consequence of acute muscle pain in the lower limb is a reduction in maximal voluntary force production (12–15). Submaximal forces up to 80% of maximum can be produced in the lower limbs during acute muscle pain, albeit with reduced endurance capacities (14, 16–19) and a greater perceived effort (6, 14). However, the impact of acute muscle pain on the ability to produce submaximal forces rapidly [i.e., rate of force development (RFD)] is unclear. Given that RFD is functionally relevant for tasks requiring rapid force generation, such as sprinting, jumping, or reacting to sudden changes in the environment (20), there is a need to determine how acute muscle pain influences this critical aspect of motor performance.

Mechanisms underpinning these motor performance changes during acute muscle pain have also been investigated using various neurophysiological measurement techniques. The interpolated twitch technique has been utilized to identify changes in voluntary activation during maximal voluntary contractions (21), with consistent decreases in voluntary activation observed during experimental quadriceps pain, suggesting that central mechanisms are responsible for decrements in physical task performance (14, 22, 23). However, current theory proposes that pain can have both excitatory and inhibitory effects on the neuromuscular system that serves as a protective mechanism to maintain muscle function while minimizing further tissue damage (11, 24, 25). In support of this, recent research using high-density surface electromyography has revealed that acute pain causes distinct adaptations throughout the motor unit pool, including the excitation of higher-threshold units and the inhibition of lower-threshold units (25). Similarly, the function of the corticospinal pathway in response to acute muscle pain has also been studied using transcranial magnetic stimulation (TMS). This produces a motor-evoked potential (MEP) of which the amplitude can reflect corticospinal excitability, and the duration of the corticospinal silent period during an active contraction reflects inhibition of the corticospinal pathway. MEP amplitudes have been shown to increase (11, 26, 27) and decrease (11, 28–30) in response to acute muscle pain, though. Inconsistent responses between studies may be influenced by the muscles tested, the presence of muscle contraction (i.e., active motor state) or the TMS stimulus intensities used. Recently, Škarabot et al. (31) demonstrated that increasing TMS intensity caused an orderly increase in the recruitment of motor units in the evoked response. Thus, based on this principle, low- and high-stimulation intensities (relative to the motor threshold) may provide further insight about the corticospinal adjustments for different populations of corticospinal neurons (low and high threshold) in response to acute muscle pain.

Research investigating the aforementioned effects of pain has used a variety of methods, such as blood flow restriction and exercise-induced muscle damage. However, these are commonly conducted contralateral to the muscle of interest, due to the changes in muscle oxygenation (32) or disruption to the excitation-contraction coupling process, which preclude the ability to study the effects of localized pain on neuromuscular function. One approach is the hypertonic saline pain model, which is primarily used in a sample of healthy participants. This method nonspecifically activates group III/IV afferents, which notably elicits an artificial pain response comparable with the experience of natural exercise-induced pain. It involves the infusion of a small bolus of hypertonic saline into the muscle (6, 13, 33). An advantage of the hypertonic saline model is that it does not appear to directly affect the contractile properties of muscle fibers (14) and can be procedurally matched with a nonpainful isotonic saline injection to serve as a control comparison (6).

Therefore, to gain further insight into the motor performance effects of localized, acute muscle pain on the lower-limb, we utilized the hypertonic saline model of muscle pain to assess RFD at different phases of the contraction along with neuromuscular function [maximal voluntary force, voluntary activation, potentiated twitch force (Q_{tw})]. In addition, to further test the hypothesis that pain has nonuniform inhibitory effects on the neuromuscular system, we investigated a variety of corticospinal responses in the painful vastus lateralis (VL) and a nonpainful synergist muscle [rectus femoris (RF)]. Specifically, we explored corticospinal excitability and inhibition across different populations of corticospinal neurons by stimulating the motor cortex at low and high TMS intensities. It was hypothesized that acute muscle pain would reduce maximal voluntary force, RFD, and voluntary activation. Furthermore, during low TMS intensities, it was hypothesized that corticospinal excitability would decrease, and corticospinal inhibition would increase, whereas during high stimulation intensities, corticospinal excitability would increase, and corticospinal inhibition would decrease for both the VL and RF.

METHODS

Participants

Fifteen healthy participants (means ± SD age: 28 ± 7 yr; height: 1.79 ± 0.08 m; mass: 86.9 ± 16.8 kg), including five females, volunteered to participate in this study. Before the commencement of testing, participants completed a physical activity readiness questionnaire and provided written informed consent. A study-specific health questionnaire was

completed to screen for contraindications to TMS (34) and intramuscular injections. Participants with neurological disorders, blood-borne diseases, a phobia of needles, any food allergies, lower limb injuries, and anyone taking medication for pre-existing pain were excluded from the study; no participants were taking analgesics at the time of the study. Participants were required to abstain from alcohol (24 h), caffeine (4 h), analgesics (6 h), and strenuous lower limb physical activity (48 h) prior to all testing. This study conformed to the standards of the Declaration of Helsinki (except for preregistration) and ethical approval was granted by the St Mary's University, Twickenham Ethics Committee (Approval Reference: SMU_ETHICS_2023-24_460). Written informed consent was provided by participants prior to their voluntary participation.

Sample Size Justification

The sample size required for the study was calculated a priori using G*Power (35). An effect size (ES) of $dz = 1.02$ from published literature (14) was used that compared the absolute maximal voluntary force in Newtons measured one minute after a hypertonic versus isotonic saline injection. To achieve 95% power ($\beta = 0.95$) with an α level of 0.05 using a two-tailed paired samples t test, a total of 15 participants were required. In addition, given the stark contrast between two interventions (pain vs. no-pain) we expected large effect sizes to be observed. Sensitivity analysis revealed that with conventional values of $\beta = 0.80$ and α of 0.05 with $n = 15$, it was possible to reliably detect Cohen's $dz = 0.78$.

Experimental Design

Following a randomized, crossover design, participants were required to attend the laboratory on three separate occasions interspaced by 3–8 days. In *visit 1*, participants' stature and body mass were measured, and they were familiarized with all experimental procedures, including an intramuscular injection of hypertonic saline, along with pain-related perceptual measures and questionnaires (see *Perceptual measures*). The following two experimental trials were completed in a randomized, incomplete-counterbalanced order. These included baseline neuromuscular function tests [electromyography (EMG), maximal compound muscle action potential (M_{max}), maximal voluntary contractions (MVC), and TMS] prior to performing the experimental procedure. Preinjection, participants performed one 4–5 s MVC to assess maximal voluntary force and RFD with a superimposed and resting peripheral nerve stimulation delivered to the femoral nerve to assess quadriceps voluntary activation and Q_{tw}. Subsequently, three sets of seven intermittent contractions at 20% of the MVC force determined at baseline were performed (~3 s on, ~3 s off) with TMS delivered at 120%, 150%, and 80/120% active motor threshold (AMT) (paired pulse, 3-ms interstimulus interval) to quantify corticospinal excitability, inhibition, and short-interval intracortical inhibition (SICI). The order of the sets was assigned randomly to each participant and counterbalanced (i.e., five participants had 120% AMT first, five had 120% second, and five had 120% third), but the order was kept consistent across trials for the same participant. A final resting peripheral nerve stimulation was delivered after the TMS to normalize corticospinal responses to. Approximately 5 min after completion of the preinjection procedures, participants received an intramuscular injection of either isotonic saline (ISO) or hypertonic (HYP) into the vastus lateralis. After the injection, participants were seated back in the isometric chair and completed the postinjection procedures, which were identical to preinjection. Temporally, the time taken from needle removal to commencement of the postinjection procedures was ~60 s, and the postinjection procedure took ~150 s. After the postinjection procedures were completed, participants completed the long-form McGill pain questionnaire. A schematic of the experimental visits can be seen in Fig. 1.

Equipment and Procedures

Experimental muscle pain.

To induce acute muscle pain, participants received an intramuscular injection of hypertonic saline while seated at rest on the edge of a medical bed in a wet laboratory <5 m from the isometric chair. In the familiarization session, a single bolus of the hypertonic saline solution [1 mL, 5.85% (B Braun Medical Industries)] was injected into the vastus lateralis (VL) of the right leg (middle third of the lateral head of the thigh between the lateral femoral condyle of the femur and the greater trochanter) while the participant's knee was at a 90° angle (33). In the experimental visits, participants received either the hypertonic saline or isotonic saline (1 mL 0.9%) injection using a 25-gauge, 25 mm, Luer-Lok, hypodermic needle (BD Microlance, Switzerland), connected to a 5-mL syringe (BD Microlance, Switzerland). Both the main researcher and participant were blinded to the injection until administration. The injection site and surrounding area were palpated and inspected to identify no local tenderness/muscle soreness before injections. All injections were administered using the Z-track method (36). Injections were performed manually for 20 s [5 s pause following needle insertion and aspiration, 10 s of solution infusion (infusion rate ~0.1 mL/s) and 5 s pause before need removal]. An aspiration was performed after the needle insertion to confirm that the needle was not in a blood vessel (14). Immediately after needle withdrawal, participants moved onto the isometric chair.

Mechanical recordings.

Participants were strapped into a custom-built isometric chair with a knee and hip angle of 90°. A Velcro strap was fastened 2 cm above the right malleoli. The strap was connected to a linear force transducer (FSB-1.5 kN Universal Cell 1.5 kN, Force Logic, Reading, UK) to measure knee extensor isometric force. A data capture module (CED Micro 1401, CED, Cambridge, UK) sampled force data onto compatible software (Signal V8, CED, Cambridge, UK), at a frequency of 2.5 kHz. Instantaneous feedback of the force traces was provided to participants on a screen directly in front of them.

Maximal voluntary contractions.

Participants were instructed to contract "as hard and as fast as possible" for 4–5 s. Three MVCs ~2 min apart were carried out at baseline to confirm participants were familiarized with MVCs, and to determine the 20% MVC force required for the subsequent TMS measurements. During the pre- and postinjection protocol, participants were required to do a single

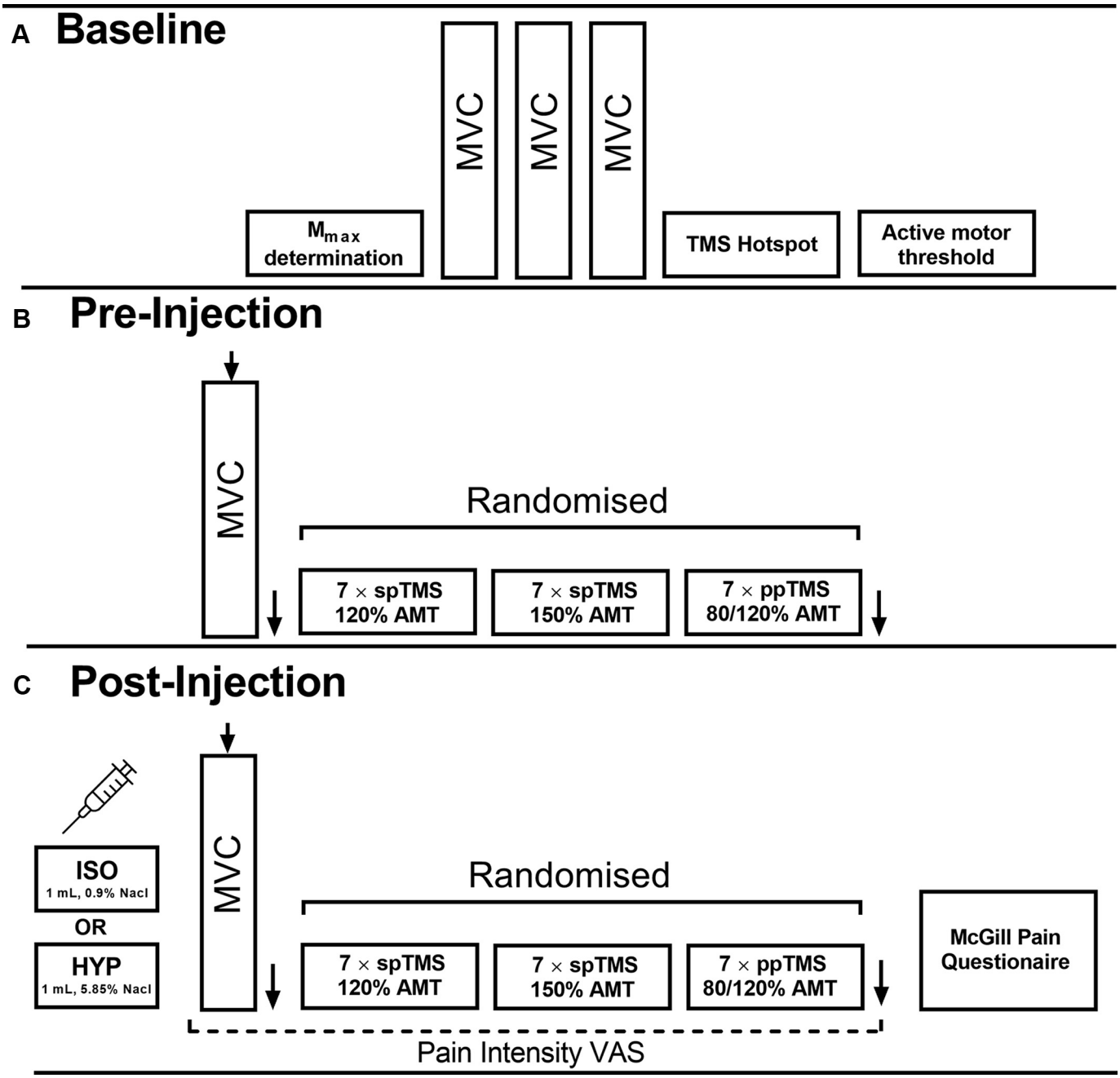

Figure 1. Schematic of the procedures during *visits 2* and *3* with the order of these trials being randomized and counterbalanced. Both visits included (*A*) baseline that involved obtaining M_{max}, determining MVC for subsequent TMS stimulations at 20% MVC. *B*: measurement of all dependent variables pre-injection. *C*: postinjection to evaluate the effects of pain induced by a hypertonic saline injection compared with the injection matched control. AMT, active motor threshold; M_{max}, maximal M-wave amplitude; MVC, maximal voluntary contraction; ppTMS, paired-pulse transcranial magnetic stimulation; spTMS, single-pulse transcranial magnetic stimulation; TMS, transcranial magnetic stimulation.

MVC. To assess voluntary activation and Q_{tw}, a peripheral nerve stimulation was delivered at peak force of the MVC and at rest (37).

Peripheral nerve stimulation.

Electrical stimulations were delivered to the femoral nerve to innervate the right quadriceps femoris using an electrical stimulator (DS7AH constant-current stimulator, Digitimer, Welwyn Garden City, UK) (maximum voltage of 400 V) that delivered a single square wave pulse (200 μs duration). Two self-adhesive 32 × 32 mm circular self-adhesive neurostimulation electrodes (Axelgaard Manufacturing, Lystrup, Denmark) were placed on the right gluteal fold (anode) and within the femoral triangle (cathode). The correct placement of the cathode was confirmed when an observable twitch response was achieved at a stimulation intensity of 100 mA. Electrical stimuli were then delivered in 20-mA increments starting from 60–100 mA, depending

on initial response, to identify the stimulation intensity that resulted in a plateau in the M-wave peak-to-peak amplitude. An additional 20% of the stimulation intensity was delivered to ensure a supramaximal stimulus was delivered (38).

Electromyography.

Muscle activity of the VL and RF was recorded using 36 mm × 36 mm bipolar surface electrodes (WhiteSensor 4500 M, Ambu Ltd., Denmark) with a 20-mm interelectrode distance. Electrode sites were identified using the SENIAM guidelines and a reference electrode was placed on the right patella. The electrode sites were shaved, abraded, and cleaned with an alcohol swab to improve conductivity (39). All EMG signals were recorded at 2.5 kHz and amplified (gain 1,000) using a signal amplifier (D440-2-Two Channel Isolated Amplifier, Digitimer, Welwyn Garden City, UK) before being recorded onto compatible software and bandpass filtered (10–1,000 Hz) (Signal V8, CED, Cambridge, UK).

Transcranial magnetic stimulation.

Using a 110-mm double-cone coil, single-pulse and paired-pulse TMS was delivered to the motor cortex with two magnetic stimulators (Magstim Bistim, The Magstim Company Ltd., Whitland, UK). The procedure started with determining the hotspot by marking on the participant, who was wearing a skintight Lycra swimming hat, the vertex, which was measured as the midpoint between the tragus and nasal inion. TMS pulses at 35%–45% maximal stimulator output were delivered during a submaximal isometric contraction (20% MVC) at 0, 1, and 2 cm anteriorly and posteriorly from the marked vertex. The anterior/posterior location that evoked the greatest MEP response was then further marked with 1 cm and 2 cm marks to the left. The greatest MEP peak-to-peak amplitude in the vastus lateralis out of all of these stimulations was defined as the hotspot. Following this, five stimulations were delivered at 45%–55% maximal stimulator output during a 20% MVC contraction and the stimulator intensity was increased or decreased in increments of 1 or 5% until the lowest intensity in which three of the five stimulations were >0.2 mV and had a visible silent period was reached. This was defined as the participant's active motor threshold (AMT). Subsequent stimulations at pre- and postinjection were set at 120% and 150% for single-pulse and 80/120% AMT for paired-pulse with an interstimulus interval of 3 ms. A stimulation intensity of 150% was selected to assess the behavior of higher-threshold corticospinal neurons compared with low-threshold that are commonly recruited during a 120% stimulation intensity (31).

Perceptual measures.

A custom-built, electronic visual analog scale (VAS) was used to record pain intensity (40) during the postinjection procedures. Participants began rating their pain once seated back in the isometric chair. The scale ranged from 0 ("no pain at all") to 100 ["extremely intense pain (almost unbearable)"]. Participants were instructed to rate their pain in relation to the worst exercise-induced pain they have previously experienced and not based on previous injuries or their worst imaginable pain. Participants adjusted the slider on the scale accordingly (see Fig. 2). After the postinjection procedures, the long-form McGill pain questionnaire was administered (41) to determine the quality of pain experienced following the injection. Participants were explicitly instructed not to include any pain from the needle stick or electrical/magnetic stimulations.

Data Analysis

All data analyses were performed in a blinded design to minimize experimenter bias. Blinding of data files was achieved by having a separate researcher code of the data files to a random number that did not correspond to the trial the participant had completed for that session. This was only revealed after data analysis was complete.

RFD was calculated from each MVC as the slope of the first 200 ms of the rising force-time curve, separated into 50-ms epochs. Contraction onset was visually determined as the last trough of the resting force trace before a rise above baseline. Force traces were observed on a consistent x- (1 s) and y-axis scale (±37.5 N around the resting force trace) on a 24.5-in. computer monitor (Acer Nitro XF252Q, Acer, New Tapei City, Taiwan). Voluntary activation was calculated with the following equation (37):

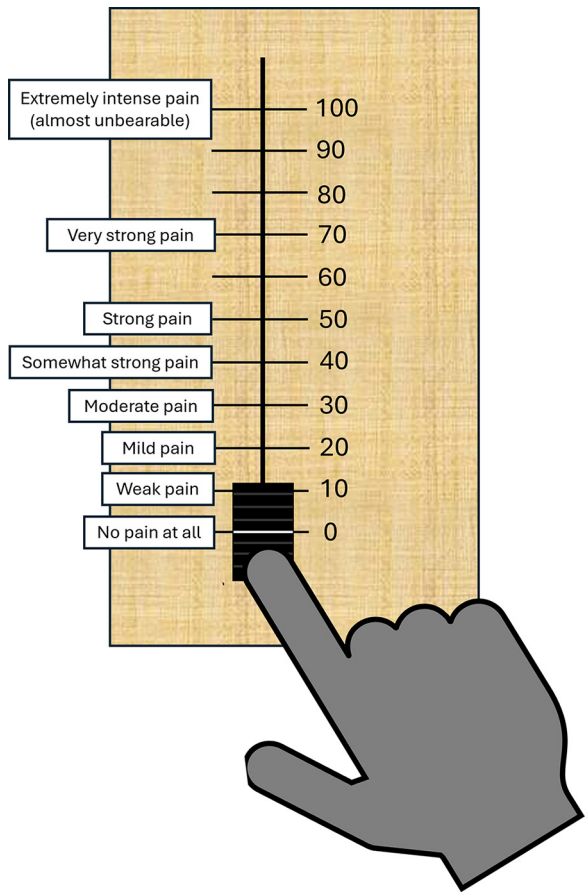

Figure 2. Illustration of the pain visual analog scale (VAS) recording device used in the present study. VAS marker position (0–100 mm) is digitally recorded in real-time. Participants adjust the slider on a moment-by-moment basis to indicate current pain intensity.

$$100 - \text{superimposed twitch force (N)} \left(\frac{\text{force before twitch (N)}}{\text{peak force (N)}} \right) \Big/ \text{resting potentiated twitch force (N)} \times 100.$$

Corticospinal excitability was determined as the average of the peak-to-peak amplitudes of the MEPs, which were then normalized to the peak-to-peak amplitude of the M_{max} (MEP·M_{max}). The duration of the TMS silent period was visually inspected from the point of the stimulus artifact to the resumption of voluntary EMG activity; the average of these durations reflected corticospinal inhibition. SICI was calculated as:

$$\left(1 - \frac{\text{mean of conditioned MEPs (mV)}}{\text{mean of unconditioned MEPs (mV)}}\right) \times 100.$$

If a lower number was observed, this reflected less inhibition, and vice versa (42). Analysis of the McGill pain questionnaire was separated into the components set out in the form. These components categorize pain into sensory (*boxes 1–10*), affective (*boxes 11–15*), evaluative (*box 16*) and miscellaneous (*boxes 17–20*). The pain intensity selected for analysis was the single value recorded by the VAS the moment before each MVC and set of stimulations was performed. A basic frequency analysis was performed to determine commonly chosen words across the sample, which was set as any word selected by more than one-third of participants.

Statistical Analysis

All statistical analyses were conducted in JAMOVI 2.5.3 (The Jamovi Project, 2024). The intensity of pain reported immediately before the MVC, TMS at 120, 150% AMT, and SICI were analyzed with a paired samples *t* test. To confirm pain ratings were not different during each of these, a one-way repeated-measures analysis of variance (ANOVA) was used to compare pain intensities in HYP only. The Δ maximal voluntary force, Q_{tw}, and voluntary activation from pre- to postinjection for ISO and HYP were analyzed with a two-tailed paired samples *t* test. Data that did not reasonably meet the assumption of normality (voluntary activation) were analyzed with a Wilcoxon signed-rank test.

RFD and TMS variables were analyzed with a repeated-measures linear mixed effects model using the "gamlj" package in JAMOVI. For RFD, contraction time (50, 100, 150, and 200 ms) and condition (ISO and HYP) were included as fixed effects. For the TMS silent period and MEP·M_{max}, stimulation intensity (120% and 150% AMT), muscle (VL and RF) and condition were included as fixed effects. For SICI, only the condition and muscle were included as fixed effects. Individual participant intercepts were included as a random effect. The preinjection values were included as a covariate to account for any between-session variability of the dependent variables, and for TMS variables, the MVC force that preceded TMS was also included as a covariate to account for any changes in the relative contraction strength that may influence corticospinal excitability/inhibition (43). Once models were fitted, normality of residuals were assessed using the Kolmogorov-Smirnov test, along with histograms and Q-Q plots. Absence of heteroscedasticity was verified by visually observing the residuals scatterplot. Variables that demonstrated heteroscedasticity (RFD) were $\log^{10}$ transformed but presented in their original scale for ease of interpretation. A simple effects analysis was performed to determine differences in each factor at each level when a statistically significant interaction effect was observed.

As subsequent exploratory analyses, we investigated whether between-pain intensity is associated with changes in key outcome variables. Pearson correlations were performed on the Δ% MVC, Δvoluntary activation, and Δ% RFD at 50 ms, as well as Δ MEP·M_{max}, Δ TMS SP, and Δ SICI measures. Correlation *P* values were corrected for multiplicity using a Holm-Bonferroni correction. Statistical significance was set at $P < 0.05$. Cohen's *d* effect sizes were reported with values of 0.2, 0.5, and 0.8 representing thresholds for small, medium, and large effects, respectively (44).

RESULTS

VAS (Pain Intensity)

There was a greater rating of pain during the MVC in HYP (31 ± 11 mm) compared with ISO (3 ± 5 mm) (mean difference = 28 mm, $t_{14} = 9.33$, $P < 0.001$, ES = 2.41). During the 120% AMT TMS, there was also greater pain ratings in HYP (33 ± 16 mm) than ISO (0 ± 1 mm) (mean difference = 33 mm, $t_{14} = 7.96$, $P < 0.001$, ES = 2.05) and during the 150% AMT TMS (HYP = 37 ± 19 mm) compared with ISO (0 ± 1 mm) (mean difference = 37 mm, $t_{14} = 7.68$, $P < 0.001$, ES = 1.98). Finally, during SICI, there was a significantly higher rating of pain in HYP (33 ± 18 mm) compared with the ISO (0 ± 0 mm) (mean difference = 33 mm, $t_{14} = 6.93$, $P < 0.001$, ES = 1.79). There was no difference in pain intensity reported during the MVC or any of the TMS measures in HYP ($F_{3,42} = 1.98$, $P = 0.132$). Individual pain intensity ratings are presented in Fig. 3.

McGill Long for Pain Questionnaire

Wilcoxon sign rank tests revealed a greater subclass rating index in HYP compared with ISO for sensory (Wilcoxon $P < 0.001$), evaluative (Wilcoxon $P = 0.014$), and miscellaneous (Wilcoxon $P = 0.003$) subclasses, but not for the affective component (Wilcoxon $P = 0.371$). Table 1 shows the

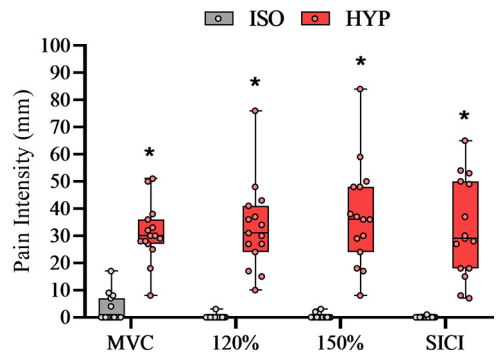

Figure 3. The absolute intensity of pain measured using a visual analog scale (VAS) following an injection of hypertonic saline (HYP) or isotonic saline (ISO) during a maximal voluntary isometric contraction (MVC) and contractions of 20% MVC during stimulations of transcranial magnetic stimulation at 120%, 150%, and short-interval intracortical inhibition (SICI) of a participant's active motor threshold. Pain intensity values presented were taken the moment before each set was performed. *Significantly different from ISO ($P < 0.001$).

Table 1. *McGill long form pain questionnaire most commonly selected words and SRI from (n = 15) participants*

	ISO	HYP
Sensory		Cramping (67%) Throbbing (47%) Aching (33%) Sharp (33%) Tender (33%)
SRI	0 [0–0.5]	12 [9–16]*
Affective		
SRI	0 [0–0]	0 [0–0]
Evaluative		
SRI	0 [0–0]	1 [0–3]*
Misc.		
SRI	0 [0–0]	2 [1–4]*

Data presented as median and interquartile range. HYP, hypertonic saline; ISO, isotonic saline; SRI, subclass rating index. *Significantly different from ISO (Wilcoxon $P < 0.05$).

median and interquartile ranges for subclass rating index values, along with the most commonly selected words by participants ($n = 15$).

Neuromuscular Function

Maximal voluntary force and RFD.

There was a greater decrease of MVC force in HYP compared with the ISO (mean difference = −73 N, $P = 0.020$, ES = −0.68). For RFD, a condition × contraction phase interaction was observed ($F_{3,96.4} = 2.76$, $P = 0.047$). Simple effects analysis revealed RFD was lower in HYP compared with ISO at 50 ms (mean difference = −313 N·s^{-1}, $P = 0.029$, ES = −0.57), 100 ms (mean difference = −617 N·s^{-1}, $P = 0.010$, ES = −0.68), and 150 ms (mean difference = −525 N·s^{-1}, $P = 0.013$, ES = −0.66) of contraction onset, but not at 200 ms (mean difference = 214 N·s^{-1}, $P = 0.406$, ES = 0.22) (Fig. 4).

Voluntary activation, Q_{tw}, and M-Wave amplitude.

There was a greater decrease of voluntary activation in HYP compared with ISO (median difference = −1.3%, $P = 0.008$, ES = −0.66). No difference in ΔQ_{tw} between HYP and ISO was observed (mean difference = −11 N, $P = 0.066$, ES = −0.51) (Fig. 5). Because Q_{tw} revealed moderate effect sizes after the MVC, we did exploratory analysis on the Q_{tw} obtained at the end of the trial (i.e., after all TMS was completed) which revealed no significant differences from ISO to HYP (median difference = −0.8 N, $P = 0.934$, ES = 0.03). M-Wave amplitude of the VL (injected muscle) was also not different between ISO and HYP (mean difference = −0.04 mV, $P = 0.844$, ES = −0.05).

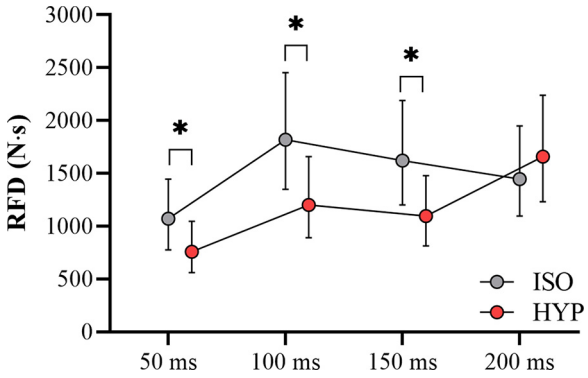

Figure 4. Rate of force development calculated as the slope over each 50-ms time period from contraction onset. *Significantly different from hypertonic saline (HYP) (interaction effect). ISO, isotonic saline; RFD, rate of force development. $n = 15$ participants.

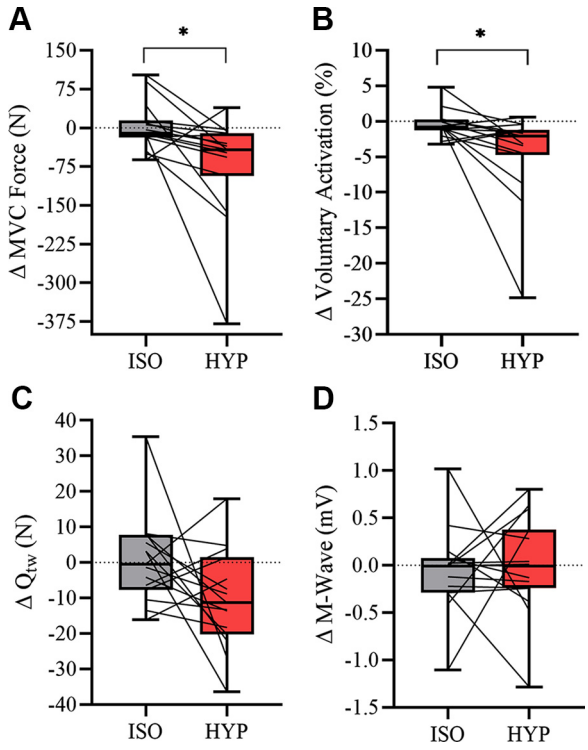

Figure 5. Change in neuromuscular function from pre- to postinjection after isotonic saline (ISO) and hypertonic saline (HYP). *A*: maximal voluntary contraction (MVC) force. *B*: voluntary activation during the MVC. *C*: quadriceps potentiated twitch force (Q_{tw}). *D*: M-Wave peak-to-peak amplitude. Data reported as box and whisker plots, with individual participants changes ($n = 15$). * significantly different from ISO ($P < 0.05$).

TMS Responses

Corticospinal excitability (MEP·M_{max}).

There was no condition × muscle × intensity ($F_{1,95.9} = 0.050$, $P = 0.824$), condition × intensity ($F_{1,96.0} = 0.539$, $P = 0.465$) or condition × muscle ($F_{1,92.2} = 0.780$, $P = 0.379$) interaction. There was a significant fixed effect of condition ($F_{1,107} = 3.354$, $P = 0.023$) with MEP·M_{max} being greater in HYP compared with ISO (mean difference = 4%, $P = 0.023$, ES = 0.60). Significant fixed effects of intensity ($F_{1,108.6} = 4.686$, $P = 0.033$) revealed MEP·M_{max} was greater in 150% AMT compared with 120% AMT (mean difference = 4%, $P = 0.033$, ES = 0.56). No effect of muscle was observed ($F_{1,108.6} = 0.68$, $P = 0.413$).

Corticospinal inhibition (TMS silent period duration).

No condition × muscle × intensity ($F_{1,97.3} = 0.005$, $P = 0.943$) or condition × muscle ($F_{1,97.3} = 0.231$, $P = 0.632$) interaction effects were observed, but there was a significant condition × intensity interaction ($F_{1,98.6} = 6.294$, $P = 0.014$). Simple effects revealed that silent period was not different between ISO and HYP at 120% AMT (mean difference = 3.1 ms, $P = 0.280$, ES = 0.28), however at 150% AMT silent period duration was shorter

in HYP compared with ISO (mean difference = −7.0 ms, P = 0.017, ES = 0.63). A significant fixed effect for intensity was observed ($F_{1,108.2}$ = 14.576, P < 0.001), with silent periods being longer at 150% AMT compared with 120% (mean difference = 9.1 ms, P < 0.001, ES = 0.99).

SICI.

There was no condition × muscle interaction ($F_{1,41.8}$ = 0.036, P = 0.851), but there was a fixed effect of condition ($F_{1,46.1}$ = 4.587, P = 0.038) where SICI was greater in HYP compared with ISO (mean difference = 8.3%, P = 0.038, ES = 0.55).

Correlations

There was no significant relationship between pain intensity and any neuromuscular outcome (all $P \geq 0.228$). Specific correlations, *r,* and *P* values can be observed in Supplemental "correlations" analysis file.

DISCUSSION

The present study aimed to assess neuromuscular function and corticospinal responses to acute muscle pain induced by an injection of hypertonic saline into the VL. The principle novel findings are as follows: *1*) muscle pain caused a significant reduction in RFD during the initial phase of the MVC. *2*) Muscle pain induced both excitatory and inhibitory responses, with an increase in MEP·M_{max} and SICI, whereas corticospinal silent period duration displayed reduced inhibition at higher stimulation intensities but was unaffected at low stimulation intensities. Furthermore, our data support previous observations (6, 13) that muscle pain reduces maximal force-generating capacity, which appears to be due to central and not peripheral mechanisms.

Perceptions of Pain Induced by Hypertonic Saline

An intramuscular injection of hypertonic saline into the vastus lateralis induced, on average, moderate (30–37/100) intensities of muscle pain (Fig. 3), which was sustained at this intensity for the entire duration of the postinjection procedures. These pain intensities are somewhat lower than what has previously been observed (45–60/100) in response to 1 mL of 5.85% NaCl injected into the vastus lateralis (16, 23, 33). One explanation for this lower pain response could be the presence of exercise-induced hypoalgesia caused by MVCs before the injections (45). In line with previous research, our results from the McGill questionnaire demonstrated a high sensory component (Table 1) from the hypertonic saline injection with commonly described words such as "aching" and "throbbing" (16, 33, 46). Affective and evaluative components were low, which was expected given the familiar, transient, and nondamaging nature of the experimental pain model.

Effects of Pain on Neuromuscular Function

Elevated muscle pain from the hypertonic saline injection caused a ~10% (large effect size) reduction in knee-extensor MVC force (Fig. 5*A*), which in agreement with the previous literature which has consistently observed a 7.5%–21% lower MVC force after an intramuscular hypertonic saline injection compared with an isotonic saline injection (12–15). In consonance with a lower MVC force in HYP was a lower voluntary activation (Fig. 5*B*), indicating that the impairment in maximal force production was due to central fatigue. It is plausible that the reduced voluntary activation is caused by a conscious disengagement from the task (i.e., reduced effort), rather than central fatigue. However, given that there were low affective and evaluative components of the pain response, it would be unlikely that the participant would have a strong reason to voluntarily apply less effort. In addition, our experimental model of pain was tonic (as opposed to movement evoked), thus the performance of the MVC would have had no immediate negative effect on the pain experience. Voluntary disengagement is likely present during movement-evoked pain, compared with tonic pain (47). Therefore, we contend that the reduced voluntary activation observed in the present study is due to a suboptimal central drive from the motor cortex.

There was no significant difference in Q_{tw} or M-Wave amplitude between trials. This further supports the possible central mechanistic involvement and absence of peripheral mechanisms during pain evoked by a hypertonic saline injection. Although a moderate effect size was observed for a lower Q_{tw} in HYP, this may be due to a small loss of potentiation from a lower absolute MVC force induced by pain (48). Indeed, when evaluating the Q_{tw} at the end of the testing procedures, where potentiation would be more consistent between trials, the effect size was trivial. Taken together, these findings support the wider literature to show that acute muscle pain induced by an intramuscular injection of hypertonic saline does not directly affect contractile function (14, 49, 50).

This is the first study to show that RFD was impaired by a moderate degree (ES = −0.57 to −0.68) in the presence of experimentally induced muscle pain, but only for the first 150 ms of the contraction (Fig. 4). It been established that the early and late components of RFD are limited by different physiological factors (51). For example, during the early RFD phase (<75 ms), maximum motor unit discharge rate is crucial for maximizing the initial force production, whereas the muscle's contractile properties have a greater influence on the latter component (52). As mentioned previously, hypertonic saline-induced acute muscle pain has no influence on the contractile properties of a muscle, which could explain why the late phase (150–200 ms) was unaffected by pain. Similar findings have been observed by Rice et al. (53), but with experimental knee pain induced by hypertonic saline injections into the infrapatellar fat pad. Motor unit discharge rates have been shown to decrease in lower threshold motor units and increase in higher threshold motor units in response to experimental muscle pain. However, it appears that this acute compensatory process was unable to preserve early-phase RFD, which presumably relies upon maximal discharge rates being achieved across the entire motor unit pool (54).

Effect of Pain on Corticospinal Responses

Corticospinal adjustments induced by hypertonic saline were assessed during low-force voluntary contractions. We

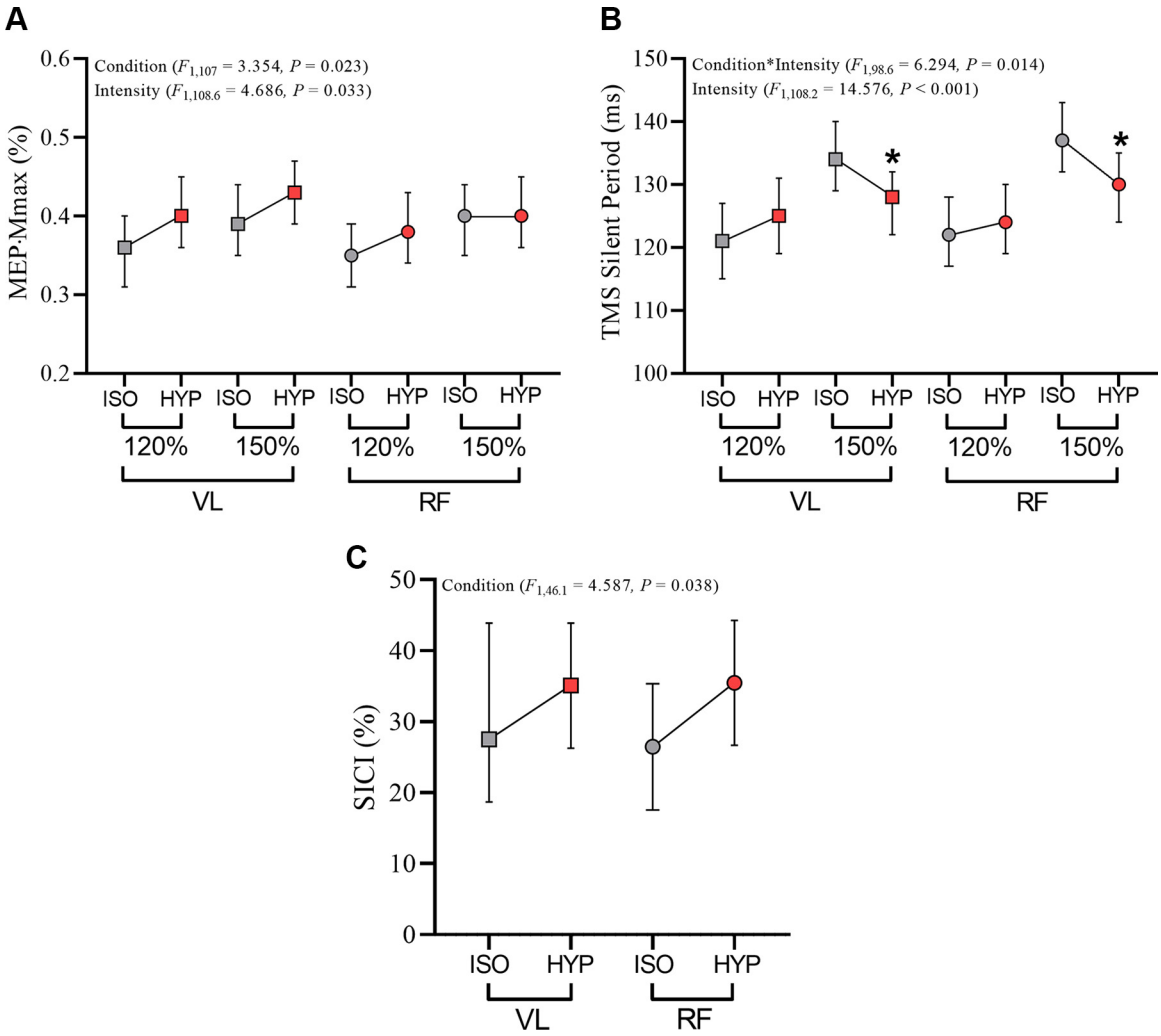

Figure 6. Transcranial magnetic stimulation (TMS) responses between isotonic saline (ISO) and hypertonic saline (HYP) postinjection. *A*: motor-evoked potential (MEP) maximal M-wave amplitude (M_{max}) indicative of corticospinal excitability. *B*: TMS silent period duration, indicative of corticospinal inhibition. *C*: short-interval intracortical inhibition, indicative of cortical inhibition. *Significantly different from ISO in 150% active motor threshold (AMT) in both vastus lateralis (VL) and rectus femoris (RF) muscles ($P < 0.05$). Data ($n = 15$) presented as estimated marginal means ± 95% confidence interval. Only significant fixed effects are presented alongside figures.

found an increase in corticospinal excitability following the hypertonic saline injection (Fig. 6*A*); which was not dependent on the stimulation intensity used, crudely suggesting increased excitability across much of the corticospinal neuronal pool, and potentially, both low and higher-threshold motor units. When assessed at rest, pain has generally been shown to cause a reduction in corticospinal excitability (55). Previous work, however, has reported an increase in the lower limb corticospinal excitability when assessed at rest during experimental knee pain (56, 57). To our knowledge, assessment of corticospinal excitability during active contractions in response to experimental lower-limb muscle pain has only previously been studied once (14). They found no differences in VL MEP amplitudes after a 1 mL hypertonic saline injection into the VL. However, it should be noted that these measurements were recorded during a fatiguing isometric knee extensor task that may introduce corticospinal adjustments in response to exercise (58) rather than solely pain. Therefore, our results are the first to demonstrate an increase in corticospinal excitability of the VL and RF in response to acute quadriceps muscle pain. Alongside increased excitation, there was evidence of reduced corticospinal inhibition during HYP that was reflected by a shorter corticospinal silent period at 150% AMT (Fig. 6*B*). Interestingly, no such difference was observed for stimulations at 120% AMT. Research by Martinez-Valdes et al. (25) found that lower-threshold motor units were able to maintain their discharge rate at both low and high contraction intensities (0%–70% MVC) compared with the higher-threshold motor units that were shown to increase discharge rate and lower their recruitment threshold. These low-threshold motor units are more susceptible to inhibitory input and are more affected by persistent inward currents (59). However, high-threshold units are not largely dependent on persistent inward currents but on increased excitatory input (60). Therefore, it is possible that our demonstration of reduced corticospinal inhibition at higher stimulation intensities was reflective of the

behavior of higher-threshold motor units, lending support to the notion that the decreased inhibition during HYP may be associated with these motor units increasing their discharge rate and lowering their recruitment threshold (25). Paired-pulse TMS measuring SICI, however, revealed an increase in inhibition (Fig. 6*C*). Given that these two measures reflect different inhibitory mechanisms (SICI; $GABA_A$ and TMS silent period duration; $GABA_B$), it is plausible that the balance of these are partly responsible for regulating motor output during pain. Although the role of each specific corticospinal adjustment is not fully understood, it has been proposed that at the system level, these adjustments allow for motor tasks to be maintained, while minimizing further pain or tissue damage (24). However, a consequence of these neurophysiological changes is a reduced maximal force-generating capacity, decreased endurance capacity and greater perceived effort (14, 61).

Physiological Relevance

It is important to note that although hypertonic saline induces sensations of pain, due to depolarization of both group III/IV nociceptors (62), there may be activation of other non-nociceptive afferents with this technique. This is due to the relatively nonspecific stimulation of free nerve endings induced by increasing extracellular sodium concentrations (63). However, the relative contribution of these non-nociceptive afferents is argued to be minor (64). Therefore, our data provide insight into the neuromuscular responses following generalized nociceptive and to a lesser degree, non-nociceptive stimulation. Interestingly, recent evidence has shown that the neuromuscular responses to group III/IV afferent stimulation may be dependent on the specific subtypes involved (e.g., nociceptive vs. non-nociceptive afferents) (65).

Given that isometric contractions themselves are sufficient to activate mechanoreceptor afferents, any additional effect from hypertonic saline on these afferents is likely minimal. Supporting this, recent work by Zambolin et al. (65) demonstrated that neuromuscular responses to mechano-nociceptive stimulation were generally directionally similar to those evoked by nonpainful mechanical stretch, with greater afferent loading—primarily driven by nociceptive input—modulating the magnitude of response. This highlights that the hypertonic saline model likely exerts its primary effect through the addition of nociceptive afferents, rather than novel mechanoreceptor recruitment.

Although recent studies have employed models such as blood flow restriction (BFR) and exercise-induced muscle damage (EIMD) to explore afferent activation and neuromuscular responses, these approaches differ in key respects from hypertonic saline infusion. BFR induces systemic cardiovascular changes and discomfort that may confound localized nociceptive input (32, 66), whereas EIMD triggers a cascade of secondary processes, including inflammation and structural muscle changes (67), that complicate the isolation of pain-related effects. In contrast, hypertonic saline provides a well-established, controllable model for inducing localized, tonic muscle pain without the accompanying metabolic or mechanical stimuli present in other paradigms (66). This allows for more precise investigation of the localized effects of nociceptive afferent activity on neuromuscular function, particularly during isometric tasks. Despite being a less "naturalistic" pain model, its internal validity and reproducibility make it highly relevant for mechanistic studies of pain and motor control.

Methodological Considerations

The interindividual variability in pain responses from participants in the present study (Fig. 3) may have resulted in heterogeneous outcomes between participants. In particular, low levels of pain reported by some participants likely introduced some uncertainty around the findings. It was not feasible to exclude these participants as this would have significantly compromised statistical power, but these findings may be limited in terms of their application to a specific pain intensity. Future studies could implement an individualized pain induction approach by varying injection volumes to evoke a desired pain intensity (e.g., "mild," "moderate," or "Strong" on the VAS). Alternatively, studies may want to assess motor performance across the descending limb of the pain response to better quantify the impact of high and low pain intensities on motor performance.

Due to the relatively short time frame of hypertonic saline-evoked pain, we were limited our protocol to 21 TMS pulses (7 pulses for 3 different stimulation parameters). Previous work has indicated that >18 pulses may be needed to obtain a "true" measure of corticospinal excitability or SICI (68). Therefore, our relatively low number of stimulations may introduce some additional variability. However, even with five stimulations, in this study we achieved within-session intraclass correlation coefficient point estimates at >0.80, which is considered "good" reliability (68, 69). Future research may want to explore additional neurophysiological measures, such as intracortical facilitation, long-interval intracortical inhibition or even spinal excitability to gain further insight into the effects of acute quadriceps muscle pain.

Five females participated in this study; however, the phases of the menstrual cycle were not controlled for. Neuromuscular function has been suggested to be reduced during the follicular phase (70); however, more recent research has found during gross motor movements, there is no change in strength and neuromuscular function for both the early and late follicular phases (71).

Conclusions

In summary, experimentally induced muscle pain in the quadriceps reduced knee extensor MVC force, RFD, and voluntary activation compared with a nonpainful isotonic saline injection. Pain exerts both excitatory and inhibitory effects on the corticospinal pathway. These findings provide further evidence to support the notion that the neuromuscular system can maintain task demands during submaximal contractions, albeit with altered excitability and inhibition, but as a consequence maximal motor task performance is impaired. These findings have implications for a wide range of individuals who experience muscle pain in the knee extensors while performing maximal and submaximal motor tasks.

DATA AVAILABILITY

The data used for statistical analysis and individual data analysis files can be found at the following link: https://doi.org/10.6084/m9.figshare.28271465.v2.

SUPPLEMENTAL MATERIAL

Supplemental File: https://doi.org/10.6084/m9.figshare.28271465.v2.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

G.E. and R.N. conceived and designed research; G.E. and R.N. performed experiments; G.E. and R.N. analyzed data; G.E., S.A.S., A.R.M., and R.N. interpreted results of experiments; G.E. and R.N. prepared figures; G.E. and R.N. drafted manuscript; G.E., S.A.S., A.R.M., and R.N. edited and revised manuscript; G.E., S.A.S., A.R.M., and R.N. approved final version of manuscript.

REFERENCES

1. **Raja SN**, **Carr DB**, **Cohen M**, **Finnerup NB**, **Flor H**, **Gibson S**, **Keefe FJ**, **Mogil JS**, **Ringkamp M**, **Sluka KA**, **Song XJ**, **Stevens B**, **Sullivan MD**, **Tutelman PR**, **Ushida T**, **Vader K.** The revised IASP definition of pain: concepts, challenges, and compromises. *Pain* 161: 1976–1982, 2020. doi:10.1097/J.PAIN.0000000000001939.
2. **Bruehl S.** Complex regional pain syndrome. *BMJ* 351: h2730, 2015. doi:10.1136/BMJ.H2730.
3. **Staud R.** Biology and therapy of fibromyalgia: pain in fibromyalgia syndrome. *Arthritis Res Ther* 8: 208, 2006. doi:10.1186/AR1950.
4. **Zambolin F**, **Duro-Ocana P**, **Faisal A**, **Bagley L**, **Gregory WJ**, **Jones AW**, **McPhee JS.** Fibromyalgia and chronic fatigue syndromes: a systematic review and meta-analysis of cardiorespiratory fitness and neuromuscular function compared with healthy individuals. *PLoS One* 17: e0276009, 2022. doi:10.1371/JOURNAL.PONE.0276009.
5. **Franklin JD**, **Atkinson G**, **Atkinson JM**, **Batterham AM.** Peak oxygen uptake in chronic fatigue syndrome/myalgic encephalomyelitis: a meta-analysis. *Int J Sports Med* 40: 77–87, 2019. doi:10.1055/A-0802-9175.
6. **O'Malley CA**, **Smith SA**, **Mauger AR**, **Norbury R.** Exercise-induced pain within endurance exercise settings: definitions, measurement, mechanisms and potential interventions. *Exp Physiol* 109: 1446–1460, 2024. doi:10.1113/EP091687.
7. **Cheung K**, **Hume PA**, **Maxwell L.** Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med* 33: 145–164, 2003. doi:10.2165/00007256-200333020-00005.
8. **Iannetta D**, **Zhang J**, **Murias JM**, **Aboodarda SJ.** Neuromuscular and perceptual mechanisms of fatigue accompanying task failure in response to moderate-, heavy-, severe-, and extreme-intensity cycling. *J Appl Physiol (1985)* 133: 323–334, 2022. doi:10.1152/JAPPLPHYSIOL.00764.2021.
9. **Spitz RW**, **Wong V**, **Bell ZW**, **Viana RB**, **Chatakondi RN**, **Abe T**, **Loenneke JP.** Blood flow restricted exercise and discomfort: a review. *J Strength Cond Res* 36: 871–879, 2022. doi:10.1519/JSC.0000000000003525.
10. **Wender CLA**, **McGranahan MJ**, **O'Connor PJ.** Exercise-induced quadriceps pain during cycling in healthy individuals: a systematic review and meta-analysis of experimental trials. *Sci Sports* 38: 827–835, 2023. doi:10.1016/J.SCISPO.2022.12.005.
11. **Martin PG**, **Weerakkody N**, **Gandevia SC**, **Taylor JL.** Group III and IV muscle afferents differentially affect the motor cortex and motoneurones in humans. *J Physiol* 586: 1277–1289, 2008. doi:10.1113/jphysiol.2007.140426.
12. **Graven-Nielsen T**, **Svensson P**, **Arendt-Nielsen L.** Effects of experimental muscle pain on muscle activity and co-ordination during static and dynamic motor function. *Electroencephalogr Clin Neurophysiol* 105: 156–164, 1997. doi:10.1016/S0924-980X(96)96554-6.
13. **Graven-Nielsen T**, **Lund H**, **Arendt-Nielsen L**, **Danneskiold-Samsøe B**, **Bliddal H.** Inhibition of maximal voluntary contraction force by experimental muscle pain: a centrally mediated mechanism. *Muscle Nerve* 26: 708–712, 2002. doi:10.1002/MUS.10225.
14. **Norbury R**, **Smith SA**, **Burnley M**, **Judge M**, **Mauger AR.** The effect of elevated muscle pain on neuromuscular fatigue during exercise. *Eur J Appl Physiol* 122: 113–126, 2022. doi:10.1007/S00421-021-04814-1.
15. **Becker K**, **Goethel M**, **Fonseca P**, **Vilas-Boas JP**, **Ervilha U.** The strategy of the brain to maintain the force production in painful contractions—a motor units pool reorganization. *Cells* 11: 3299, 2022. doi:10.3390/CELLS11203299.
16. **Smith SA**, **Micklewright D**, **Winter SL**, **Mauger AR.** Muscle pain induced by hypertonic saline in the knee extensors decreases single-limb isometric time to task failure. *Eur J Appl Physiol* 120: 2047–2058, 2020. doi:10.1007/S00421-020-04425-2.
17. **Canestri R**, **Franco-Alvarenga PE**, **Brietzke C**, **Vinícius Í**, **Smith SA**, **Mauger AR**, **Goethel MF**, **Pires FO.** Effects of experimentally induced muscle pain on endurance performance: a proof-of-concept study assessing neurophysiological and perceptual responses. *Psychophysiology* 58: e13810, 2021. doi:10.1111/PSYP.13810.
18. **Zhang J**, **Abel S**, **Macphail M**, **Aboodarda SJ.** Persistent contralateral pain compromises exercise tolerance but does not alter corticomotor responses during repeated, submaximal isometric knee extensions to task failure. *Neuroscience* 526: 267–276, 2023. doi:10.1016/J.NEUROSCIENCE.2023.07.005.
19. **Aboodarda SJ**, **Iannetta D**, **Emami N**, **Varesco G**, **Murias JM**, **Millet GY.** Effects of pre-induced fatigue vs. concurrent pain on exercise tolerance, neuromuscular performance and corticospinal responses of locomotor muscles. *J Physiol* 598: 285–302, 2020. doi:10.1113/JP278943.
20. **Maffiuletti NA.** Physiological and methodological considerations for the use of neuromuscular electrical stimulation. *Eur J Appl Physiol* 110: 223–234, 2010. doi:10.1007/S00421-010-1502-Y.
21. **Prevc P**, **Misotic N**, **Stirn I**, **Tomazin K.** Perceived discomfort and voluntary activation of quadriceps muscle assessed with interpolated paired or triple electrical stimuli. *Int J Environ Res Public Health* 20: 4799, 2023. doi:10.3390/IJERPH20064799.
22. **Finn H**, **Rouffet DM**, **Kennedy DS**, **Green S**, **Taylor JL.** Motoneuron excitability of the quadriceps decreases during a fatiguing submaximal isometric contraction. *J Appl Physiol (1985)* 124: 970–979, 2018. doi:10.1152/JAPPLPHYSIOL.00739.2017.
23. **Norbury R**, **Smith SA**, **Burnley M**, **Judge M**, **Mauger AR.** The effect of hypertonic saline evoked muscle pain on neurophysiological changes and exercise performance in the contralateral limb. *Exp Brain Res* 240: 1423–1434, 2022. doi:10.1007/S00221-022-06342-6.
24. **Hodges PW**, **Tucker K.** Moving differently in pain: a new theory to explain the adaptation to pain. *Pain* 152: S90–S98, 2011. doi:10.1016/J.PAIN.2010.10.020.
25. **Martinez-Valdes E**, **Negro F**, **Farina D**, **Falla D.** Divergent response of low- versus high-threshold motor units to experimental muscle pain. *J Physiol* 598: 2093–2108, 2020. doi:10.1113/JP279225.
26. **Del Santo F**, **Gelli F**, **Spidalieri R**, **Rossi A.** Corticospinal drive during painful voluntary contractions at constant force output. *Brain Res* 1128: 91–98, 2007. doi:10.1016/J.BRAINRES.2006.09.039.
27. **Schabrun SM**, **Christensen SW**, **Mrachacz-Kersting N**, **Graven-Nielsen T.** Motor cortex reorganization and impaired function in the transition to sustained muscle pain. *Cereb Cortex* 26: 1878–1890, 2016. doi:10.1093/CERCOR/BHU319.
28. **Farina S**, **Valeriani M**, **Rosso T**, **Aglioti S**, **Tamburin S**, **Fiaschi A**, **Tinazzi M.** Transient inhibition of the human motor cortex by capsaicin-induced pain. A study with transcranial magnetic stimulation. *Neurosci Lett* 314: 97–101, 2001. doi:10.1016/S0304-3940(01)02297-2.
29. **Le Pera D**, **Graven-Nielsen T**, **Valeriani M**, **Oliviero A**, **Di Lazzaro V**, **Tonali PA**, **Arendt-Nielsen L.** Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. *Clin Neurophysiol* 112: 1633–1641, 2001. doi:10.1016/S1388-2457(01)00631-9.
30. **Summers SJ**, **Chipchase LS**, **Hirata R**, **Graven-Nielsen T**, **Cavaleri R**, **Schabrun SM.** Motor adaptation varies between individuals in the transition to sustained pain. *Pain* 160: 2115–2125, 2019. doi:10.1097/J.PAIN.0000000000001604.
31. **Škarabot J**, **Ammann C**, **Balshaw TG**, **Divjak M**, **Urh F**, **Murks N**, **Foffani G**, **Holobar A**, **Holobar A.** Decoding firings of a large

population of human motor units from high-density surface electromyogram in response to transcranial magnetic stimulation. *J Physiol* 601: 1719–1744, 2023. doi:10.1113/JP284043.
32. **Zambolin F**, **Duro Ocana P**, **Goulding R**, **Sanderson A**, **Venturelli M**, **Wood G**, **McPhee J**, **Parr JVV.** The corticomuscular response to experimental pain via blood flow occlusion when applied to the ipsilateral and contralateral leg during an isometric force task. *Psychophysiology* 61: e14466, 2024. doi:10.1111/PSYP.14466.
33. **Smith SA**, **Norbury R**, **Hunt AJ**, **Mauger AR.** Intra- and interindividual reliability of muscle pain induced by an intramuscular injection of hypertonic saline injection into the quadriceps. *Eur J Pain* 27: 1216–1225, 2023. doi:10.1002/EJP.2151.
34. **Rossi S**, **Hallett M**, **Rossini PM**, **Pascual-Leone A.** Screening questionnaire before TMS: an update. *Clin Neurophysiol* 122: 1686, 2011. doi:10.1016/J.CLINPH.2010.12.037.
35. **Faul F**, **Erdfelder E**, **Lang AG**, **Buchner A.** G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 39: 175–191, 2007. doi:10.3758/BF03193146.
36. **Pullen RL Jr.** Administering medication by the Z-track method. *Nursing* 35: 24, 2005. doi:10.1097/00152193-200507000-00018.
37. **Strojnik V**, **Komi PV.** Neuromuscular fatigue after maximal stretch-shortening cycle exercise. *J Appl Physiol (1985)* 84: 344–350, 1998. doi:10.1152/JAPPL.1998.84.1.344.
38. **Osborne JO**, **Tallent J**, **Girard O**, **Marshall PW**, **Kidgell D**, **Buhmann R.** Neuromuscular electrical stimulation during maximal voluntary contraction: a Delphi survey with expert consensus. *Eur J Appl Physiol* 123: 2203–2212, 2023. doi:10.1007/S00421-023-05232-1.
39. **Li G**, **Wang S**, **Duan YY.** Towards conductive-gel-free electrodes: Understanding the wet electrode, semi-dry electrode and dry electrode-skin interface impedance using electrochemical impedance spectroscopy fitting. *Sens Actuators B Chem* 277: 250–260, 2018. doi:10.1016/J.SNB.2018.08.155.
40. **Cook DB**, **O'Connor PJ**, **Eubanks SA**, **Smith JC**, **Lee M.** Naturally occurring muscle pain during exercise: assessment and experimental evidence. *Med Sci Sports Exerc* 29: 999–1012, 1997. doi:10.1097/00005768-199708000-00004.
41. **Melzack R.** The short-form McGill pain questionnaire. *Pain* 30: 191–197, 1987. doi:10.1016/0304-3959(87)91074-8.
42. **Lackmy A**, **Marchand-Pauvert V.** The estimation of short intra-cortical inhibition depends on the proportion of spinal motoneurones activated by corticospinal inputs. *Clin Neurophysiol* 121: 612–621, 2010. doi:10.1016/J.CLINPH.2009.12.011.
43. **Oya T**, **Hoffman BW**, **Cresswell AG.** Corticospinal-evoked responses in lower limb muscles during voluntary contractions at varying strengths. *J Appl Physiol (1985)* 105: 1527–1532, 2008. doi:10.1152/JAPPLPHYSIOL.90586.2008.
44. **Fritz CO**, **Morris PE**, **Richler JJ.** Effect size estimates: current use, calculations, and interpretation. *J Exp Psychol Gen* 141: 2–18, 2012. doi:10.1037/a0024338.
45. **Bement MK**, **Dicapo J**, **Rasiarmos R**, **Hunter SK.** Dose response of isometric contractions on pain perception in healthy adults. *Med Sci Sports Exerc* 40: 1880–1889, 2008. doi:10.1249/MSS.0B013E31817EEECC.
46. **Graven-Nielsen T**, **McArdle A**, **Phoenix J**, **Arendt-Nielsen L**, **Jensen TS**, **Jackson MJ**, **Edwards RH.** In vivo model of muscle pain: quantification of intramuscular chemical, electrical, and pressure changes associated with saline-induced muscle pain in humans. *Pain* 69: 137–143, 1997. doi:10.1016/S0304-3959(96)03270-8.
47. **Cabral HV**, **Devecchi V**, **Oxendale C**, **Jenkinson N**, **Falla D**, **Gallina A.** Effect of movement-evoked and tonic experimental pain on muscle force production. *Scand J Med Sci Sports* 34: e14509, 2024. doi:10.1111/SMS.14509.
48. **Eon P**, **Jubeau M**, **Cattagni T.** Post-activation potentiation after isometric contractions is strongly related to contraction intensity despite the similar torque–time integral. *Exp Physiol* 109: 915–925, 2024. doi:10.1113/EP091700.
49. **Farina D**, **Arendt-Nielsen L**, **Graven-Nielsen T.** Experimental muscle pain decreases voluntary EMG activity but does not affect the muscle potential evoked by transcutaneous electrical stimulation. *Clin Neurophysiol* 116: 1558–1565, 2005. doi:10.1016/J.CLINPH.2005.03.009.
50. **Sousa MV**, **Goethel M**, **Becker KM**, **Diefenthaeler F**, **Fernandes RJ**, **de Santana Toro Batista I**, **Vilas-Boas JP**, **Ervilha U.** Effect of experimentally induced muscle pain on neuromuscular control of force production. *Hum Mov Sci* 95: 103219, 2024. doi:10.1016/J.HUMOV.2024.103219.
51. **Aagaard P**, **Simonsen EB**, **Andersen JL**, **Magnusson P**, **Dyhre-Poulsen P.** Increased rate of force development and neural drive of human skeletal muscle following resistance training. *J Appl Physiol (1985)* 93: 1318–1326, 2002. doi:10.1152/JAPPLPHYSIOL.00283.2002.
52. **D'Emanuele S**, **Tarperi C**, **Rainoldi A**, **Schena F**, **Boccia G.** Neural and contractile determinants of burst-like explosive isometric contractions of the knee extensors. *Scand J Med Sci Sports* 33: 127–135, 2023. doi:10.1111/SMS.14244.
53. **Rice DA**, **Mannion J**, **Lewis GN**, **McNair PJ**, **Fort L.** Experimental knee pain impairs joint torque and rate of force development in isometric and isokinetic muscle activation. *Eur J Appl Physiol* 119: 2065–2073, 2019. doi:10.1007/S00421-019-04195-6.
54. **Del Vecchio A**, **Negro F**, **Holobar A**, **Casolo A**, **Folland JP**, **Felici F**, **Farina D.** You are as fast as your motor neurons: speed of recruitment and maximal discharge of motor neurons determine the maximal rate of force development in humans. *J Physiol* 597: 2445–2456, 2019. doi:10.1113/JP277396.
55. **Chowdhury NS**, **Chang WJ**, **Millard SK**, **Skippen P**, **Bilska K**, **Seminowicz DA**, **Schabrun SM.** The effect of acute and sustained pain on corticomotor excitability: a systematic review and meta-analysis of group and individual level data. *J Pain* 23: 1680–1696, 2022. doi:10.1016/J.JPAIN.2022.04.012.
56. **Rice DA**, **Graven-Nielsen T**, **Lewis GN**, **McNair PJ**, **Dalbeth N.** The effects of experimental knee pain on lower limb corticospinal and motor cortex excitability. *Arthritis Res Ther* 17: 204–208, 2015. doi:10.1186/S13075-015-0724-0.
57. **Rice DA**, **Lewis GN**, **Graven-Nielsen T**, **Luther R**, **McNair PJ.** Experimental hand and knee pain cause differential effects on corticomotor excitability. *J Pain* 22: 789–796, 2021. doi:10.1016/J.JPAIN.2021.01.006.
58. **Goodall S**, **Howatson G**, **Thomas K.** Modulation of specific inhibitory networks in fatigued locomotor muscles of healthy males. *Exp Brain Res* 236: 463–473, 2018. doi:10.1007/S00221-017-5142-X.
59. **Mesquita RNO**, **Škarabot J**, **Pearcey GEP.** Low-threshold motor units can be a pain during experimental muscle pain. *J Physiol* 598: 2545–2547, 2020. doi:10.1113/JP279872.
60. **Martinez-Valdes E**, **Negro F**, **Arvanitidis M**, **Farina D**, **Falla D.** Pain-induced changes in motor unit discharge depend on recruitment threshold and contraction speed. *J Appl Physiol (1985)* 131: 1260–1271, 2021. doi:10.1152/JAPPLPHYSIOL.01011.2020.
61. **O'Malley CA**, **Norbury R**, **Smith SA**, **Fullerton CL**, **Mauger AR.** Elevated muscle pain induced by a hypertonic saline injection reduces power output independent of physiological changes during fixed perceived effort cycling. *J Appl Physiol (1985)* 137: 99–110, 2024. doi:10.1152/JAPPLPHYSIOL.00325.2023.
62. **Mense S.** Algesic agents exciting muscle nociceptors. *Exp Brain Res* 196: 89–100, 2009. doi:10.1007/S00221-008-1674-4.
63. **Hoheisel U**, **Unger T**, **Mense S.** Excitatory and modulatory effects of inflammatory cytokines and neurotrophins on mechanosensitive group IV muscle afferents in the rat. *Pain* 114: 168–176, 2005. doi:10.1016/J.PAIN.2004.12.020.
64. **Korotkov A**, **Ljubisavljevic M**, **Thunberg J**, **Kataeva G**, **Roudas M**, **Pakhomov S**, **Radovanovic S**, **Lyskov E**, **Medvedev S**, **Johansson H.** Changes in human regional cerebral blood flow following hypertonic saline induced experimental muscle pain: a positron emission tomography study. *Neurosci Lett* 335: 119–123, 2002. doi:10.1016/S0304-3940(02)01181-3.
65. **Zambolin F**, **Laginestra FG**, **Favaretto T**, **Giuriato G**, **Ottaviani MM**, **Schena F**, **Duro-Ocana P**, **McPhee JS**, **Venturelli M.** Activation of skeletal muscle mechanoreceptors and nociceptors reduces the exercise performance of the contralateral homologous muscles. *Am J Physiol Regul Integr Comp Physiol* 327: R389–R399, 2024. doi:10.1152/AJPREGU.00069.2024.
66. **Graven-Nielsen T**, **Jansson Y**, **Segerdahl M**, **Kristensen JD**, **Mense S**, **Arendt-Nielsen L**, **Sollevi A.** Experimental pain by ischaemic contractions compared with pain by intramuscular infusions of adenosine and hypertonic saline. *Eur J Pain* 7: 93–102, 2003. doi:10.1016/S1090-3801(02)00069-1.

67. **Goodall S**, **Thomas K**, **Barwood M**, **Keane K**, **Gonzalez JT**, **St Clair Gibson A**, **Howatson G.** Neuromuscular changes and the rapid adaptation following a bout of damaging eccentric exercise. *Acta Physiol (Oxf)* 220: 486–500, 2017. doi:10.1111/APHA.12844.
68. **Brownstein CG**, **Ansdell P**, **Škarabot J**, **Howatson G**, **Goodall S**, **Thomas K.** An optimal protocol for measurement of corticospinal excitability, short intracortical inhibition and intracortical facilitation in the rectus femoris. *J Neurol Sci* 394: 45–56, 2018. doi:10.1016/J.JNS.2018.09.001.
69. **Koo TK**, **Li MY.** A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med* 15: 155–163, 2016 [Erratum in *J Chiropr Med* 16: 346, 2017]. doi:10.1016/J.JCM.2016.02.012.
70. **Weidauer LEE**, **Zwart MB**, **Clapper J**, **Albert JOE**, **Vukovich M**, **Specker B.** Neuromuscular performance changes throughout the menstrual cycle in physically active females. *J Musculoskelet Neuronal Interact* 20: 314–324, 2020.
71. **Dragutinovic B**, **Moser F**, **Notbohm HL**, **Ihalainen JK**, **Bloch W**, **Schumann M.** Influence of menstrual cycle and oral contraceptive phases on strength performance, neuromuscular fatigue and perceived exertion. *J Appl Physiol (1985)* 137: 919–933, 2024. doi:10.1152/japplphysiol.00198.2024.