Title: Hypertonic Saline-Evoked Muscle Pain in the Quadriceps Reduces Neuromuscular Performance and Alters Corticospinal Excitability

Running Head: Muscle pain and neuromuscular function

Authors: Gabriella Elder[1], Samuel Smith[*2], Alexis Mauger [2], Ryan Norbury [1,3]

[1] Faculty of Sport, Technology and Health Sciences, St Mary's University, Twickenham, UK.

[2] School of Natural Sciences, University of Kent, Canterbury, UK,

[3] Department of Kinesiology, University of Wisconsin-Whitewater, WI, USA

* Corresponding Author:

Dr Samuel A Smith

S.A.Smith-75@kent.ac.uk

ORCiD: 0000-0002-0833-0878

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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 non-painful 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 to ISO. Corticospinal excitability increased in HYP compared to 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 to 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.

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New and 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 non-uniform effects of pain on the corticospinal tract.

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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 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 on to 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

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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 utilised 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 which serves as a protective mechanism to maintain muscle function whilst minimising further tissue damage (24–26). In support of this, recent research using high-density surface electromyography has revealed that pain causes distinct adaptations throughout the motor unit pool, including the excitation of higher-threshold units and the inhibition of lower-threshold units (26). 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 (25, 27, 28) and decrease (25, 29–31) in response to acute muscle pain though. Inconsistent responses between studies may be influenced by the muscles tested,

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the presence of muscle contraction (i.e., active motor state) or the TMS stimulus intensities used. Recently, Škarabot et al. (32) 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 have 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 (33) or disruption to excitation-contraction coupling process, which preclude the ability to study the effects of *localised* pain on neuromuscular function. One approach is the hypertonic saline pain model, which is primarily used in a sample of healthy participants. This method non-specifically activates group III/IV afferents, which notably elicits an artificial pain response comparable with the experience of natural exercise-induced pain, involves the infusion of a small bolus of hypertonic saline into the muscle (6, 13, 34). An advantage of the hypertonic saline model is that it does not appear to directly affect the contractile properties of muscle fibres (14) and can be procedurally matched with a non-painful isotonic saline injection to serve as a control comparison (6).

Therefore, to gain further insight into the motor performance effects of localised, acute muscle pain on the lower-limb, we utilised 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}]). Additionally, to further test the hypothesis that pain has non-uniform inhibitory effects on the neuromuscular system, we investigated a variety of corticospinal responses in the painful vastus lateralis (VL) and a non-painful synergist muscle (rectus femoris [RF]). Specifically,

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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 hypothesised that acute muscle pain would reduce maximal voluntary force, RFD, and voluntary activation. Furthermore, during low TMS intensities, it was hypothesised 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 (mean ± SD age: 28 ± 7 years; height: 1.79 ± 0.08 m; mass: 86.9 ± 16.8 kg), including 5 females, volunteered to participate in this study. Prior to 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 (35) 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 pre-registration) and ethical approval was granted by the St Mary's University, Twickenham Ethics Committee (approval reference: SMU_ETHICS_2023-24_460).

Sample Size Justification

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The sample size required for the study was calculated a-priori using G*Power (36). An effect size of $dz = 1.02$ from published literature (14) was used which 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 alpha level of 0.05 using a two-tailed paired samples t-test, a total of fifteen participants were required. Additionally, given the stark contrast between two interventions (pain vs no-pain) we expected large effect sizes to be observed. Sensitivity analysis reveals that normal conventions of $\beta = 0.80$ and alpha of 0.05 with $n = 15$, it was possible to reliably detect Cohen's $dz = 0.78$.

Experimental Design

Following a randomised, crossover design, participants were required to attend the laboratory on three separate occasions interspaced by 3-8 days. In visit one, participants' stature and body mass were measured, and they were familiarised 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 randomised, 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. Pre-injection, 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 inter-stimulus interval) to quantify corticospinal excitability, inhibition and short interval

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196 intracortical inhibition (SICI). The order of the sets was assigned randomly to each
197 participant and counterbalanced (i.e., five participants had 120% AMT first, five had 120%
198 second and five had 120% third), but the order was kept consistent across trials for the same
199 participant. A final resting peripheral nerve stimulation was delivered after the TMS to
200 normalise corticospinal responses to. Approximately five minutes after completion of the pre-
201 injection procedures, participants received an intramuscular injection of either isotonic saline
202 (ISO) or hypertonic (HYP) into the vastus lateralis. After the injection, participants were
203 seated back in the isometric chair and completed the post-injection procedures, which were
204 identical to pre-injection. Temporally, the time taken from needle removal to commencement
205 of the post-injection procedures was approximately 60 s and the post-injection procedure took
206 approximately 150 seconds. After the post-injection procedures were completed, participants
207 completed the long-form McGill pain questionnaire. A schematic of the experimental visits
208 can be seen in Figure 1.
209
210 Equipment and Procedures
211 *Experimental Muscle Pain.* To induce acute muscle pain, participants received an
212 intramuscular injection of hypertonic saline while seated at rest on the edge of a medical bed
213 in a wet laboratory < 5 m from the isometric chair. In the familiarisation session a single
214 bolus of the hypertonic saline solution (1 mL, 5.85% [B Braun Medical Industries]) was
215 injected into the vastus lateralis (VL) of the right leg (middle third of the lateral head of the
216 thigh between the lateral femoral condyle of the femur and the greater trochanter) while the
217 participant's knee was at a 90º angle (34). In the experimental visits, participants received
218 either the hypertonic saline or isotonic saline (1 mL 0.9%) injection using a 25-gauge, 25
219 mm, Luer-Lok, hypodermic needle (BD Microlance, Switzerland), connected to a 5 mL
220 syringe (BD Microlance, Switzerland). Both the main researcher and participant were blinded

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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 (37). Injections were performed manually for 20 seconds (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 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 90º. 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 seconds. Three MVCs ~ 2 minutes apart were carried out at baseline to confirm participants were familiarised with MVCs, and to determine the 20% MVC force required for the subsequent TMS measurements. During the pre- and post-injection protocol, participants were required to do a single MVC. To assess voluntary activation and Q_{tw}, a peripheral nerve stimulation was delivered at peak force of the MVC and at rest (38).

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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 (39).

Electromyography. Muscle activity of the VL and RF was recorded using 36 mm × 36 mm bipolar surface electrodes (WhiteSensor 4500M, 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 (40). All EMG signals were recorded at 2.5 kHz and amplified (gain 1000) using a signal amplifier (D440-2-Two Channel Isolated Amplifier, Digitimer, Welwyn Garden City, UK) before being recorded onto compatible software and bandpass filtered (10-1000 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

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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 which 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 out 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 post-injection were set at 120% and 150% for single-pulse and 80/120% AMT for paired-pulse with an inter stimulus interval of 3 ms. A stimulation intensity of 150% was selected to assess the behaviour of higher-threshold corticospinal neurons in comparison to low-threshold that are commonly recruited during a 120% stimulation intensity (32).

Perceptual Measures. A custom-built, electronic visual analogue scale (VAS) was used to record pain intensity (41) during the post-injection 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 figure 2). After the post-injection procedures, the long-form McGill pain questionnaire was administered (42) to determine the quality of pain

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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 analysis were performed in a blinded design to minimise experimenter bias. Blinding of data files was achieved by having a separate researcher code 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-inch computer monitor (Acer Nitro XF252Q, Acer, New Tapei City, Taiwan). Voluntary activation was calculated with the following equation (38):

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

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

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

If a lower number was observed, this reflected less inhibition, and vice versa (43). Analysis of the McGill pain questionnaire was separated into the components set out in the form. These components categorise pain into sensory (boxes 1 – 10), affective (boxes 11-15),

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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 prior to the MVC, TMS at 120, 150% AMT, and SICI were analysed 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 post injection for ISO and HYP were analysed with a two-tailed paired samples t-test. Data which did not reasonably meet the assumption of normality (voluntary activation) were analysed with a Wilcoxon sign rank test.

RFD and TMS variables were analysed 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·Mmax, stimulation intensity (120% and 150% AMT), muscle (VL and RF) and condition were included as fixed effects. For SICI, only condition and muscle were included as fixed effects. Individual participant intercepts were included as a random effect. The pre-injection values were included as a covariate to account for any between-session variability of the dependent variables, and for TMS variables, the MVC force which preceded TMS was also included as a covariate to account for any changes in the relative contraction strength which may influence corticospinal excitability/inhibition (44). Once models were

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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 which 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 associated with changes in key outcome variables. Pearson correlations were performed on the Δ% MVC, Δ VA and Δ% RFD at 50 ms, as well as Δ MEP·Mmax, 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 (Fritz et al., 2012).

Results

VAS (Pain Intensity)

There was a greater rating of pain during the MVC in HYP (31 ± 11 mm) compared to 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) as well as during the 150% AMT TMS (HYP = 37 ± 19 mm) compared to ISO (0 ± 1 mm) (mean difference = 37 mm, $t_{14} = 7.68$, $P < 0.001$, ES = 1.98). Lastly, during SICI, there was a significantly higher rating of pain in HYP (33 ± 18 mm) compared to 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

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MVC, or any of the TMS measures in HYP ($F_{3,42}$ = 1.98, P = 0.132). Individual pain intensity ratings are presented in Figure 3.

McGill long for pain questionnaire

	ISO	HYP
Sensory	-	Cramping (67%)
		Throbbing (47%)
		Aching (33%)
		Sharp (33%)
		Tender (33%)
SRI	0 [0 – 0.5]	12 [9 – 16]*

Wilcoxon sign rank tests revealed a greater subclass rating index in HYP compared to 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 median and interquartile ranges for subclass rating index values, along with most commonly selected words by participants.

Table 1. McGill long form pain questionnaire most commonly selected words and subclass rating index (SRI). Data presented as median and interquartile range.

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Affective	-	-
SRI	0 [0 – 0]	0 [0 – 0]
Evaluative	-	-
SRI	0 [0 – 0]	1 [0 – 3]*
Misc.	-	-
SRI	0 [0 – 0]	2 [1 – 4]*

*denotes significantly different from ISO (Wilcoxon $P < 0.05$).

Neuromuscular Function

Maximal Voluntary Force and RFD. There was a greater decrease of MVC force in HYP compared to 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 to ISO at 50 ms (mean difference = -313 $N \cdot s^{-1}$, $P = 0.029$, ES = -0.57), 100 ms (mean difference = -617 $N \cdot s^{-1}$, $P = 0.010$, $ES = -0.68$) and 150 ms (mean difference = -525 $N \cdot s^{-1}$, $P = 0.013$, ES = -0.66) of contraction onset, but not at 200 ms (mean difference = 214 $N \cdot s^{-1}$, $P = 0.406$, ES = 0.22)(Figure 4).

Voluntary Activation, Q_{tw} and M-Wave Amplitude. There was a greater decrease of VA in HYP compared to 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) (figure 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).

TMS Responses

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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 to 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 to 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 to 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 to 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 to ISO (mean difference = 8.3%, $P = 0.038$, ES = 0.55).

Figure 6. TMS responses between ISO and HYP post-injection. a. MEP·M_{max} indicative of corticospinal excitability. b. TMS Silent period duration, indicative of corticospinal inhibition. c. Short interval intracortical inhibition, indicative of cortical inhibition. * denotes significantly different from ISO in 150% AMT in both VL and RF muscles ($P < 0.05$). Data

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presented as estimated marginal mean ± 95% confidence interval. Only significant fixed effects are presented alongside figures.

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 supplementary 'correlations' analysis file.

Discussion

The aim of the present study was 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 i.) Muscle pain caused a significant reduction in RFD during the initial phase of the MVC. ii.) 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 supports 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 (figure 3), which was sustained at this intensity for the entire duration of the post-injection procedures. These pain intensities are somewhat lower what has previously been observed (45-60/100) in response to 1 mL of 5.85% NaCl injected into the vastus lateralis (16, 23, 34). One explanation for this lower pain response could be the presence of exercise-induced hypoalgesia caused by MVCs prior to the

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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, 34, 46). Affective and evaluative components were low, which was expected given the familiar, transient, and non-damaging 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 (figure 5a), 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 to an isotonic saline injection (12–15). In consonance with a lower MVC force in HYP was a lower voluntary activation (figure 5b), 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. Additionally, 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 to 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. Whilst a moderate effect

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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 the peripheral fatigue (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 (Figure 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 maximising 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. (2019), 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 found an increase in corticospinal excitability following the

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hypertonic saline injection (figure 6a); 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 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 which 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 quadriceps acute muscle pain. Alongside increased excitation, there was evidence of reduced corticospinal inhibition during HYP which was reflected by a shorter corticospinal silent period at 150% AMT (figure 6b). Interestingly, no such difference was observed for stimulations at 120% AMT. Research by Martinez-Valdes et al. (26) found that lower-threshold motor units were able to maintain their discharge rate at both low and high contraction intensities (0 – 70% MVC) compared to 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 behaviour of higher-threshold motor units, lending support to the notion that the decreased inhibition during HYP may be associated

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with these motor units increasing their discharge rate and lowering their recruitment threshold (26). Paired-pulse TMS measuring SICI, however, revealed an increase in inhibition (figure 6c). Given that these two measures reflect different inhibitory mechanisms, (SICI; GABAa and TMS silent period duration; GABAb) it is plausible that the balance of these are partly responsible for regulating motor output during pain. Whilst 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, whilst minimising 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 whilst hypertonic saline induces sensations of pain, due to depolarisation 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 non-specific 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 generalised 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 sub-types 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

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neuromuscular responses to mechano-nociceptive stimulation were generally directionally similar to those evoked by non-painful 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.

While 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 localised nociceptive input (33, 66), while 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 localised, tonic muscle pain without the accompanying metabolic or mechanical stimuli present in other paradigms (66). This allows for more precise investigation of the localised 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 inter-individual variability in pain responses from participants in the present study (figure 3) may have resulted in heterogenous 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

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of their application to a specific pain intensity. Future studies could implement an individualised 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 5 stimulations, in this study we achieved within-session ICC 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 (64); however, more recent research has found during gross motor movements, these is no change in strength and neuromuscular function for both the early and late follicular phases (71).

Conclusion

In summary, experimentally induced muscle pain in the quadriceps reduced knee extensor MVC force, RFD and voluntary activation compared to a non-painful 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

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602 can maintain task demands during submaximal contractions, albeit with altered excitability
603 and inhibition, but as a consequence maximal motor task performance is impaired. These
604 findings have implications for a wide range of individuals who experience muscle pain in the
605 knee extensors whilst performing maximal and submaximal motor tasks.
606
607
608 **Additional Information**

609 Data Availability Statement: The data used for statistical analysis and individual data analysis
610 files can be found at the following link: https://doi.org/10.6084/m9.figshare.28271465.v1

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618 responsible for conception and design of the work, acquisition, analysis, and interpretation of
619 data for the work, as well as drafting the work and revising it critically for important
620 intellectual content. ARM and SAS were responsible for interpretation of data for the work
621 and for revising it critically for important intellectual content. All authors approved the final
622 version of the manuscript; agree to be accountable for all aspects of the work in ensuring that
623 questions related to the accuracy or integrity of any part of the work are appropriately

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investigated and resolved; and all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Figure Captions

Figure 1. Schematic of the procedures during visits two and three with the order of these trials being randomised and counterbalanced. Both visits included a. baseline which involved obtaining M_{max}, determining MVC for subsequent TMS stimulations at 20% MVC. b. Measurement of all dependent variables pre-injection. c. post-injection to evaluate the effects of pain induced by a hypertonic saline injection compared to the injection matched control. Mmax = maximal M-wave amplitude; MVC = maximal voluntary contraction; TMS = transcranial magnetic stimulation; spTMS = single pulse transcranial magnetic stimulation; ppTMS = paired pulse transcranial magnetic stimulation. AMT = active motor threshold.

Figure 2. Illustration of the pain 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.

Figure 3. The absolute intensity of pain measured using a visual analogue 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

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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. * denotes significantly different from ISO ($P < 0.001$).

Figure 4. Rate of force development calculated as the slope over each 50 ms time period from contraction onset. * denotes significantly different from HYP (interaction effect).

Figure 5. Change in neuromuscular function from pre- to post-injection after ISO and HYP. a. Maximal voluntary contraction 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 changes.

Figure 6. TMS responses between ISO and HYP post-injection. a. MEP·M_{max} indicative of corticospinal excitability. b. TMS Silent period duration, indicative of corticospinal inhibition. c. Short interval intracortical inhibition, indicative of cortical inhibition. * denotes significantly different from ISO in 150% AMT in both VL and RF muscles ($P < 0.05$). Data presented as estimated marginal mean ± 95% confidence interval. Only significant fixed effects are presented alongside figures.

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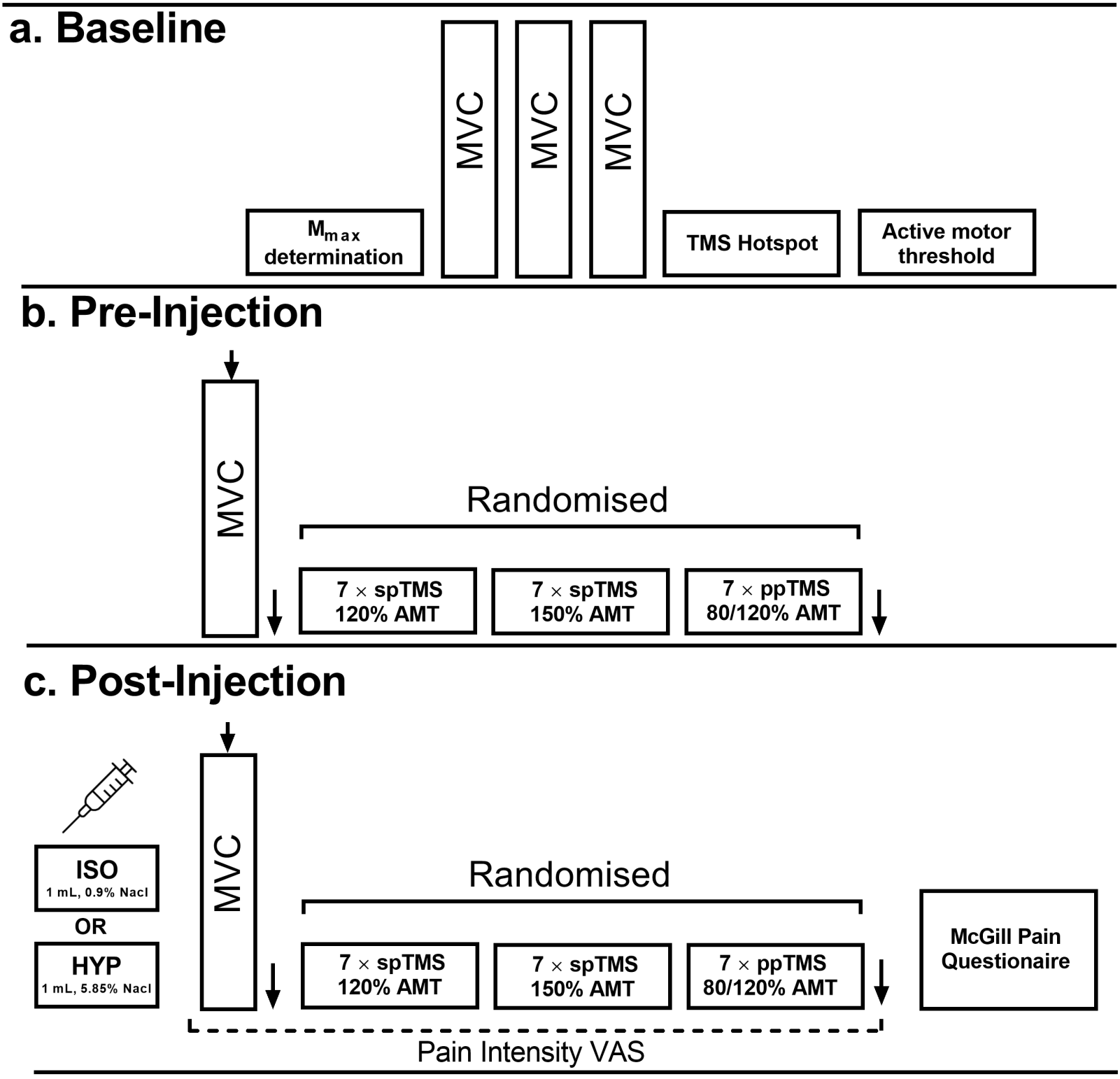

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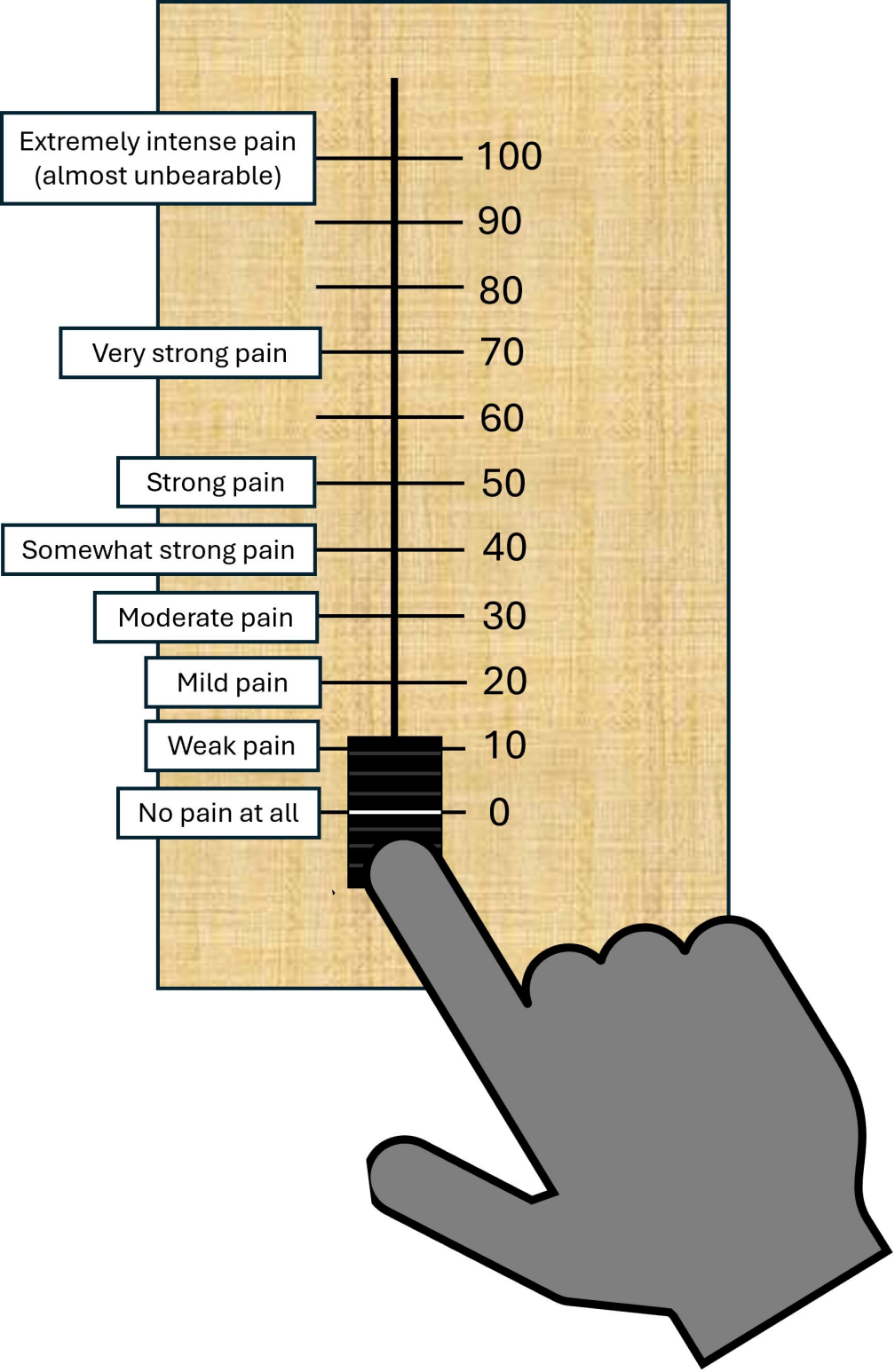

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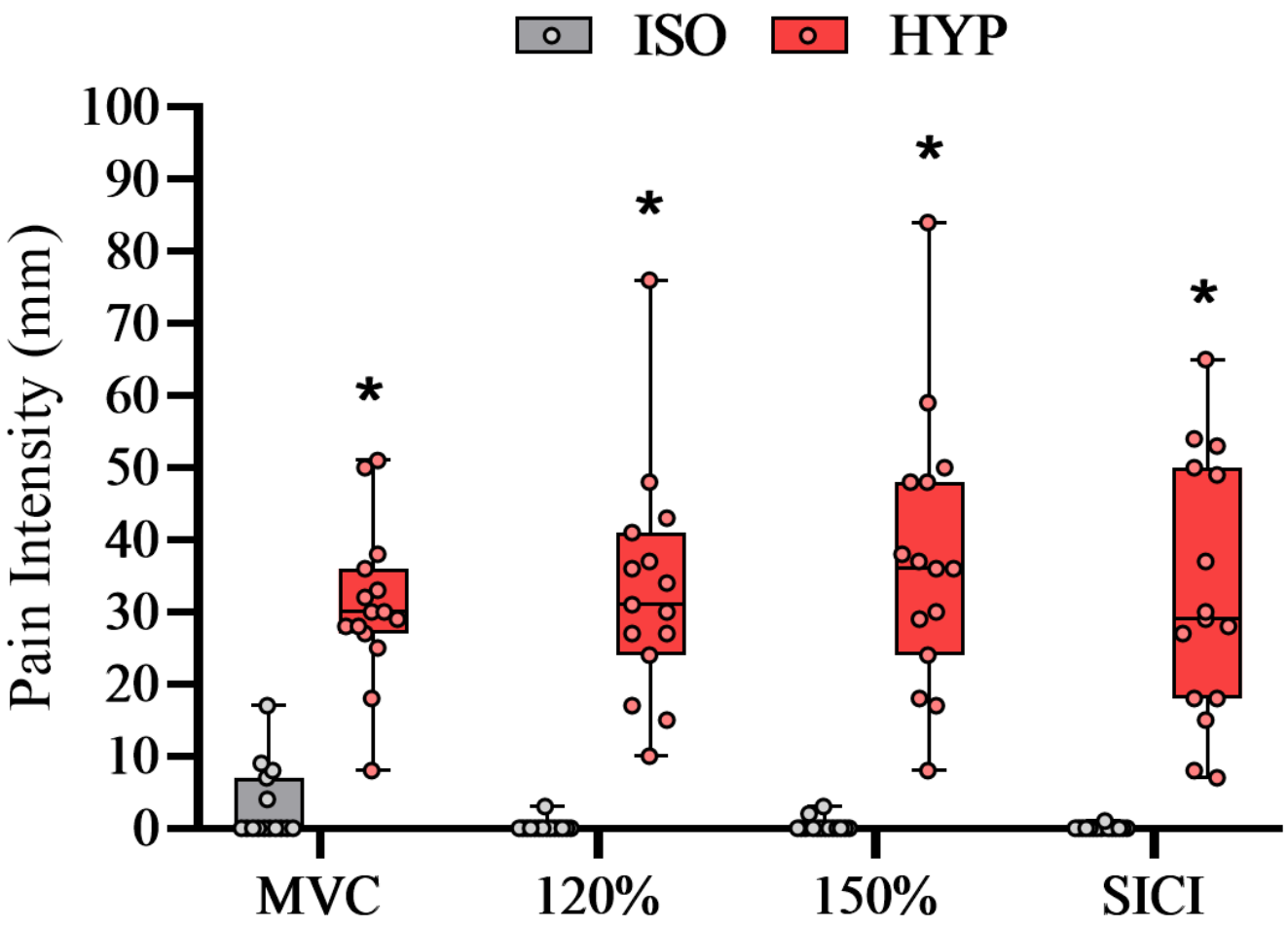

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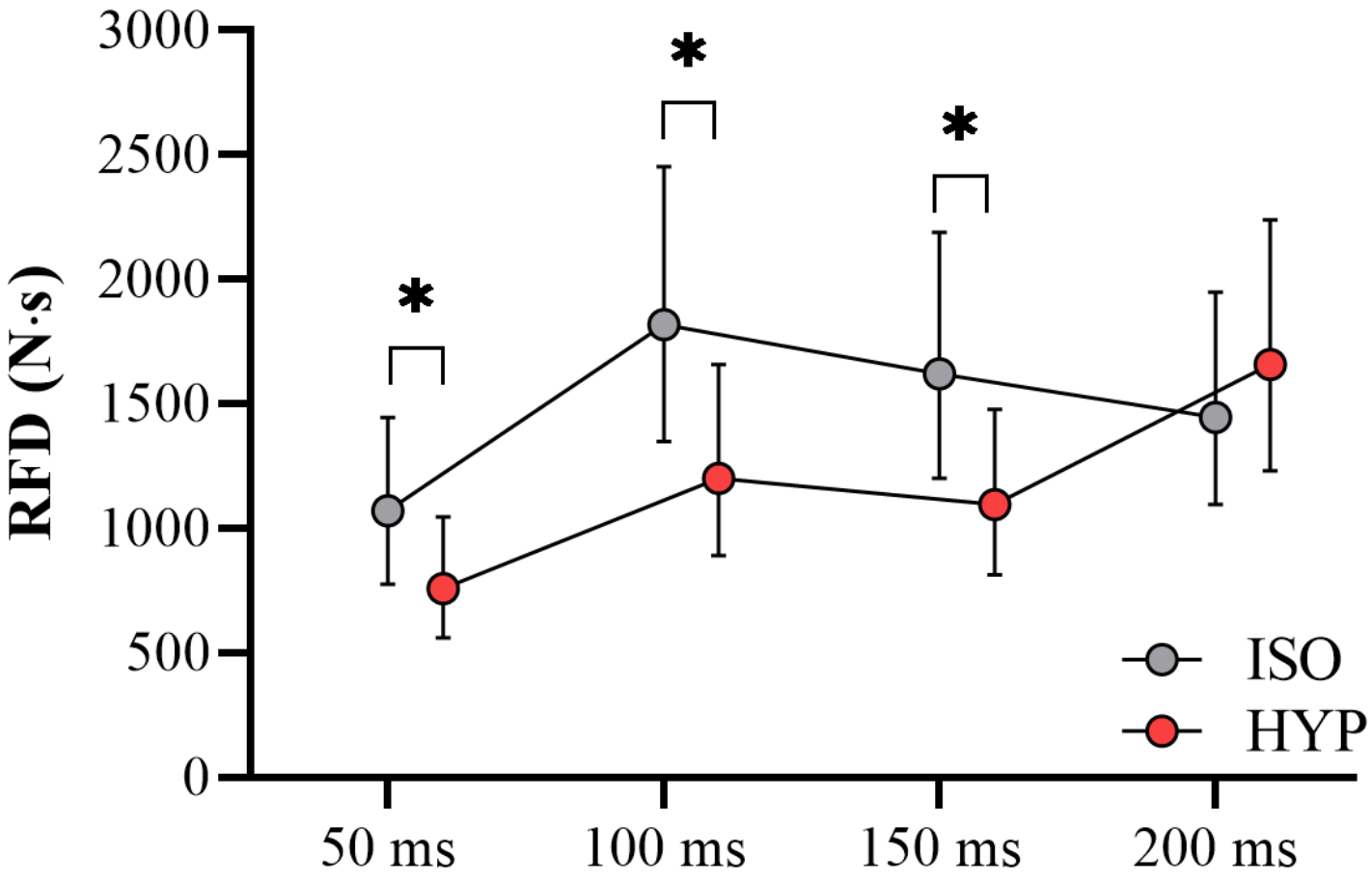

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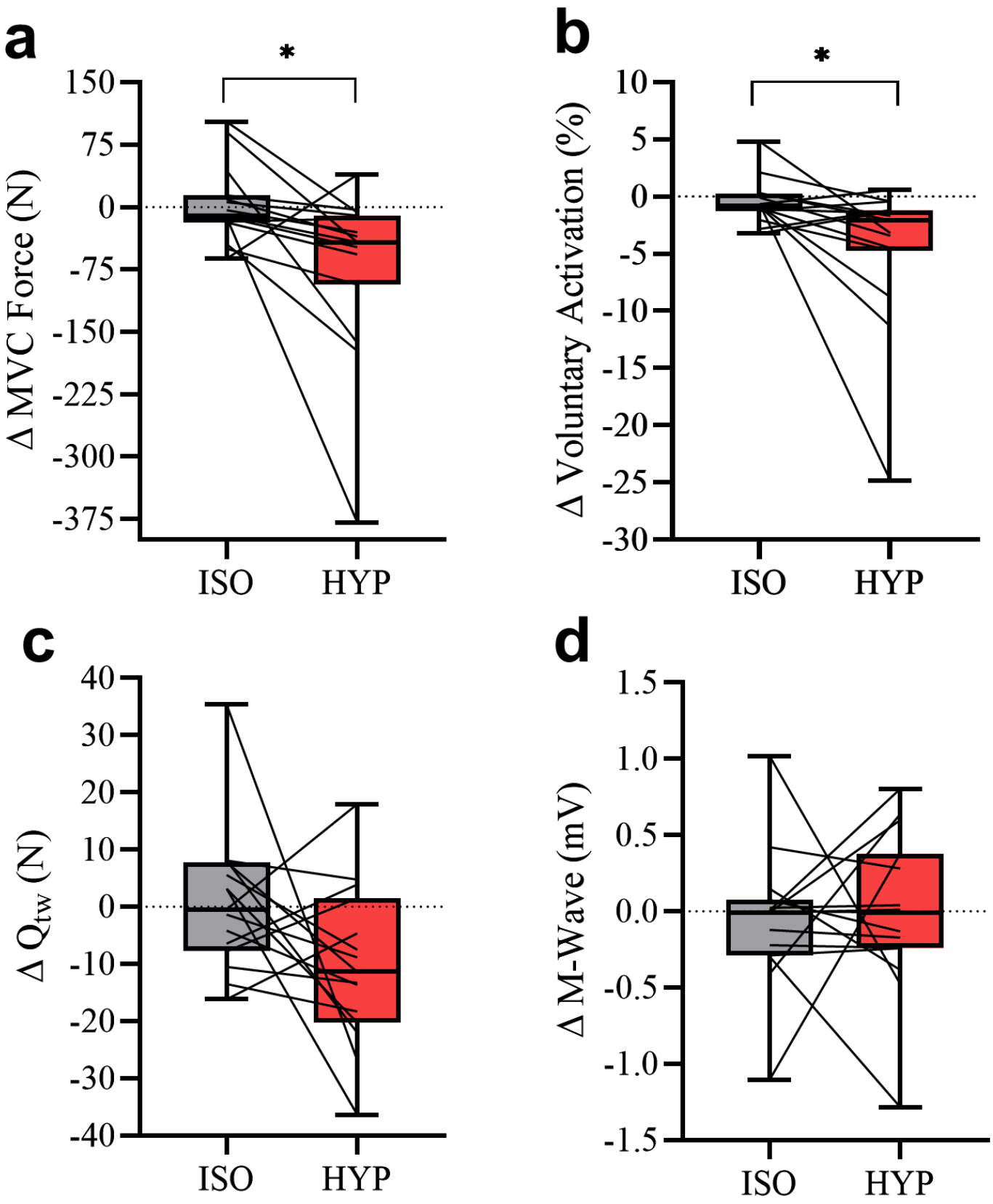

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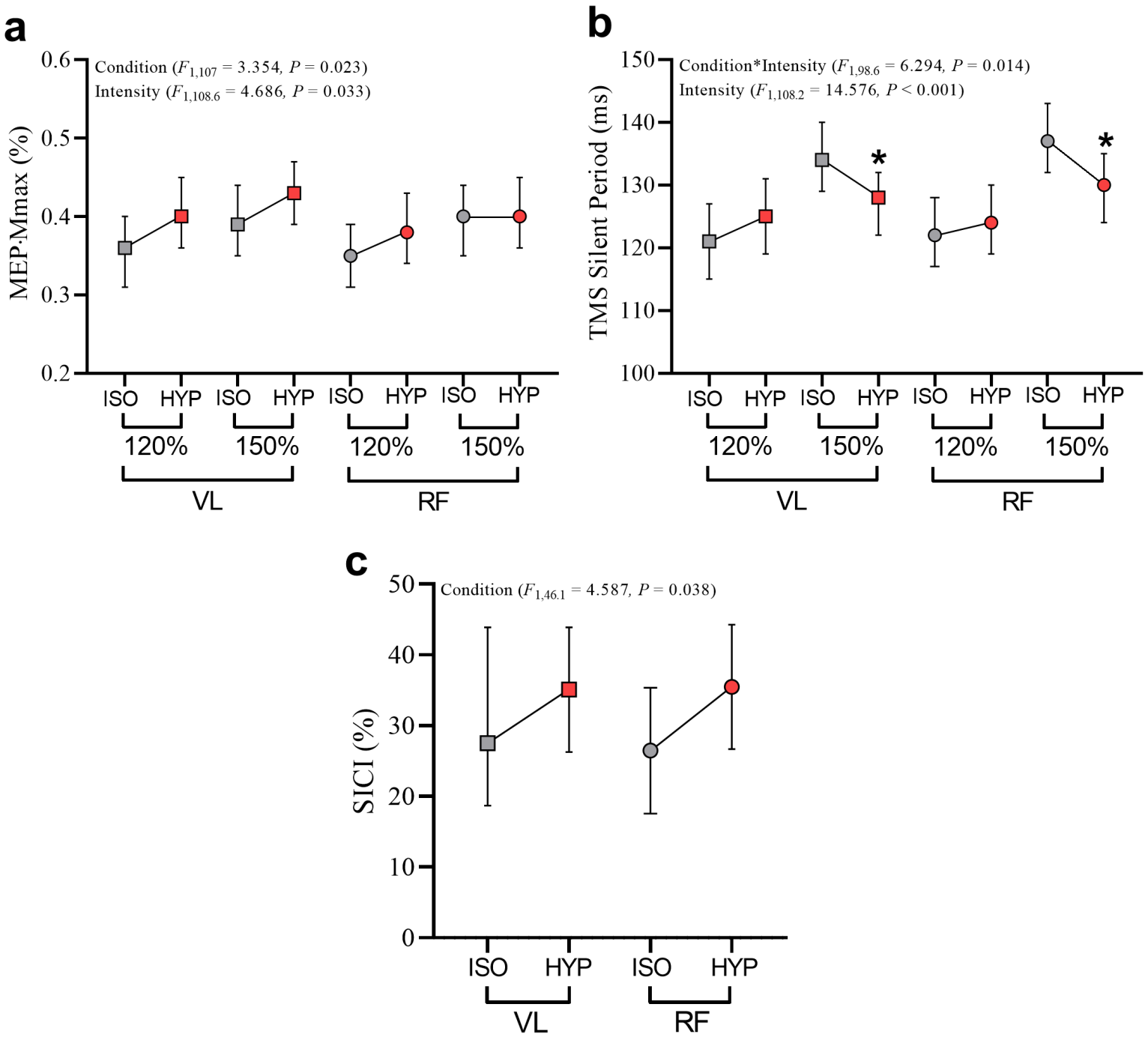

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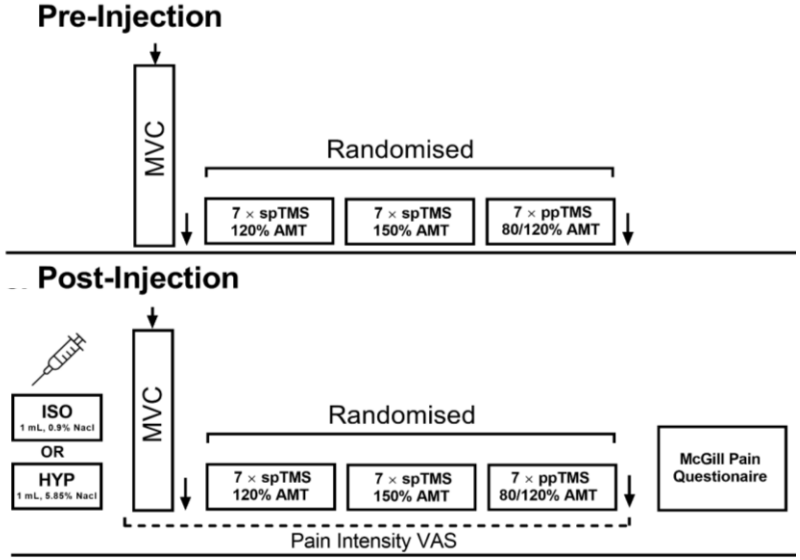
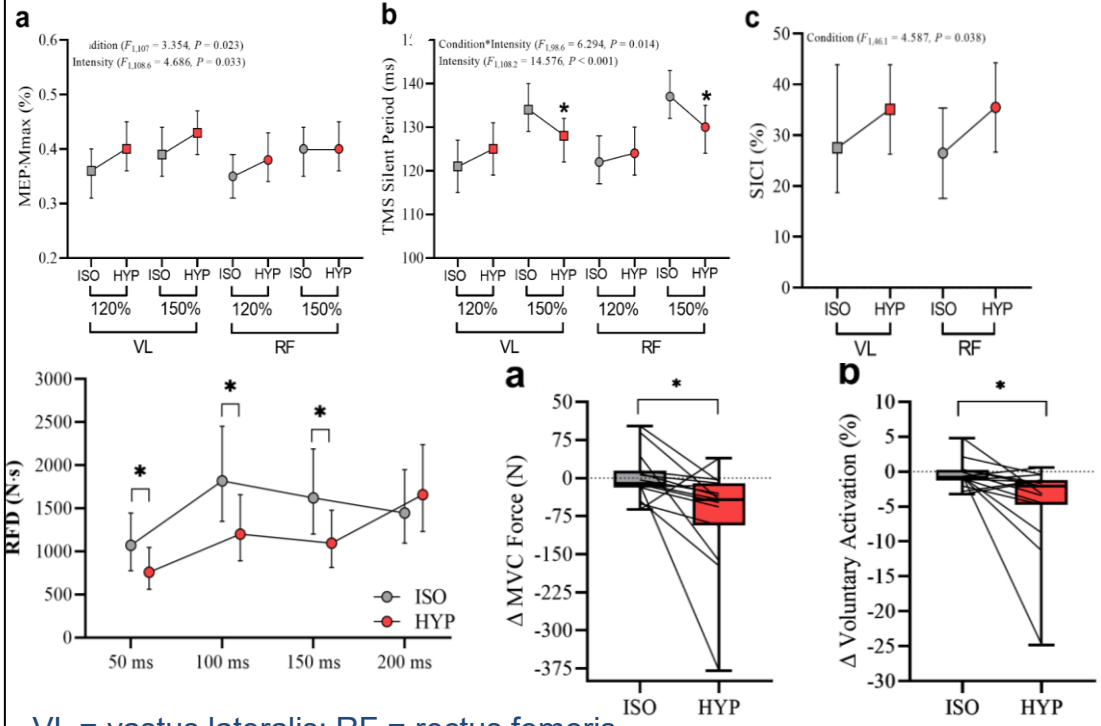
Experimental muscle pain reduces quadriceps neuromuscular function and alters corticospinal excitability.

Methods



Randomised, within-subject design.
MVC = maximal voluntary contraction; Sp and pp TMS = single/paired pulse transcranial magnetic stimulation.

Outcomes



VL = vastus lateralis; RF = rectus femoris

Conclusion

- Increased pain from a hypertonic saline injection (HYP) reduced maximal voluntary force and voluntary activation compared to a non-painful isotonic saline injection (ISO).
- Rate of force development was significantly slower from 50 – 150 ms in the painful, hypertonic saline condition.
- Corticospinal responses show both excitatory and inhibitory responses, with some evidence to suggest stimulation intensity dependent effects.

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