MUSCLE PAIN FROM AN INTRAMUSCULAR INJECTION OF HYPERTONIC SALINE INCREASES VARIABILITY IN KNEE EXTENSOR TORQUE REPRODUCTION

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Author Contributions

SAS and ARM were responsible for the conception and design of the study, and data acquisition. SAS, DM, SLW, and ARM were responsible for data analysis and interpretation. SAS was responsible for drafting the manuscript. SAS, DM, SLW and ARM were responsible for critically revising and editing intellectual content.

Running head: Muscle pain increases variability in torque reproduction

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ABSTRACT

Purpose: The intensity of exercise-induced pain (EIP) reflects the metabolic environment in the exercising muscle, so during endurance exercise this may inform the intelligent regulation of work rate. Conversely, the acute debilitating effects of EIP on motor unit recruitment could impair the estimation of force produced by the muscle and impair judgement of current exercise intensity. This study investigated whether muscle pain that feels like EIP, administered via intramuscular injection of hypertonic saline, interferes with the ability to accurately reproduce torque in a muscle group relevant to locomotive exercise. **Methods**: On separate days, fourteen participants completed an isometric torque reproduction task of the knee extensors. Participants were required to produce torque at 15 and 20% maximal voluntary torque (MVIT), without visual feedback before (Baseline), during (Pain/No Pain), and after (Recovery) an injection of 0.9% isotonic saline (Control) or 5.8% hypertonic saline (Experimental) into the vastus lateralis of the right leg. **Results:** An elevated reported intensity of pain, and a significantly increased variance in mean contraction torque at both 15% (P=0.049) and 20% (P=0.002) MVIT was observed in the Experimental compared to the Control condition. Both 15 and 20% target torques were performed at a similar pain intensity in the Experimental condition (15% MVIT, 4.2 ± 1.9; 20% MVIT, 4.5 ± 2.2; P>0.05). **Conclusion:** These findings demonstrate that the increased muscle pain from the injection of hypertonic saline impeded accurate reproduction of knee extensor torque. These findings have implications for the detrimental impact of EIP on exercise regulation and endurance performance.

New & Noteworthy

We provide novel data demonstrating that the presence of muscle pain interferes with estimations of torque produced by the knee extensors, which could impair judgement of work-rate during endurance exercise. The novelty of our study is in the application of the hypertonic saline experimental model into a quadriceps muscle during short, submaximal isometric contractions at an intensity that provides a more translatable assessment of the impact of exercise-induced pain on work-rate regulation during whole-body exercise.

Key words: Nociception, Exercise Regulation, Proprioception, Effort perception, Pain

INTRODUCTION

Exercise-induced pain (EIP) increases linearly with exercise intensity and duration (9), and has been suggested to provide useful sensory feedback about the relative strain of exercising muscles (7, 27, 31). During intense and fatiguing muscle contractions, nociceptors of Group III and IV muscle afferents become sensitised and activated by an accumulation of metabolites which induce the perception of EIP, but are also implicated in peripheral fatigue and the description of its perception (31, 38). Resultantly, EIP is often associated with other physiological and psychological factors of fatigue, and has been suggested to independently exacerbate or contribute to the development of fatigue (27). A change in muscle torque complexity, which is suggested to reflect the adaptability of the neuromuscular system and is reduced during fatiguing maximal and submaximal isometric contractions (34), could provide a non-invasive method to evaluate the fatiguing effect of EIP.

During whole-body exercise, sensations of EIP may facilitate conscious control of homeostatic disturbance during exercise by enabling the intelligent regulation of available energetic resources (i.e. pacing) (12, 27, 54). However, the relationship between EIP and fatigue is likely more complex since it also causes various acute debilitating effects associated with motor unit recruitment (17) and, as a protective mechanism, restricts movement to reduce pain. Consequentially, whilst EIP may provide insight about the metabolic environment in the exercising muscle, these potentially detrimental adaptations may reduce the accuracy of estimations of work done and/or force applied by the muscle, which could impair pacing decisions during whole-body exercise.

Supressing the unpleasant sensations associated with intense exercise may allow a higher exercise-intensity to be tolerated and sustained (28), however near complete removal of this

information via spinal afferent blockade appears to impair the exerciser's ability to select and maintain a physiologically optimal work rate (3). Spinal blockade studies show the importance of Group III and IV afferents to the performance of whole-body exercise (2, 3) but reveal less about the parallel effects of nociception and perceived pain on other systems such as cardiovascular control.

Intramuscular hypertonic saline injection produces a muscle pain that feels like the naturally occurring EIP experienced during intense exercise (16, 50), and is therefore a useful method to investigate how EIP affects self-regulation of exercise intensity. This technique has previously been used in contralateral limb-matching tasks to assess the impact of tonic muscle pain on the judgement of torque in small muscle groups (40, 41, 57). In these studies, increased pain impeded the ability to accurately match torque, with pain intensity and degree of error correlating such that participants consistently overestimated the force generated by the painful muscle.

This experimental approach could, however, be confounded by potential differences between the contralateral limbs (1, 36). To provide a more translatable assessment of the impact of EIP on whole-body exercise, the relationship between muscle pain and the reproduction of isometric torque production should be evaluated in the larger muscle groups of the lower limb such as the knee extensors, which have an important and fundamental role in the generation of force during locomotion and exercise.

As such, the aim of the present study was to ascertain whether experimentally induced muscle pain in the vastus lateralis (VL) using an intramuscular injection of hypertonic saline would affect the ability to accurately gauge the torque produced by the knee extensor muscles

126 in a single-limb isometric torque reproduction task. We tested the hypothesis that
127 experimental muscle pain in the VL reduces torque reproduction accuracy (as quantified by
128 the variance in mismatch between target and actual torque) of low intensity isometric
129 contractions when compared to a placebo control condition.
130
131 **METHODS**
132 ***Ethical Approval***
133 All procedures and protocols were approved by the School of Sport and Exercises (University
134 of Kent) Research Ethics Advisory Group (Prop 140_2016_17) in conformity with the
135 Declaration of Helsinki, and its later amendments or comparable ethical standards. All
136 participants were informed of the study experimental procedures, and written informed
137 consent was obtained to confirm participation.
138
139 ***Participants***
140 Fourteen healthy and recreationally active participants (13 male, 1 female; mean ± SD: age,
141 25.3 ± 4.5 years; height 1.78 ± 0.1 m; body mass 73.9 ± 12.3 kg; physical activity 5.6 ± 2.2
142 hours per week) volunteered to participate in the present study. Assuming a statistical power
143 of 0.8 at an alpha level of 0.05, the sample size was estimated using G*Power software (13)
144 based on the effect size reported in a similar study in our laboratory using hypertonic saline
145 injections (50). All participants attended each visit in a similar psychological state as assessed
146 by the Positive and Negative Affect Schedule (PANAS) (56), which was completed at the
147 start of each visit.
148
149 Before each visit, participants were instructed to refrain from vigorous exercise (24 h) and
150 abstain from the consumption of alcohol (48 h), analgesics (6 h) and caffeine (8 h).

151 Participants with existing knee pain, cardiorespiratory disease, neurological disorders, blood
152 borne viruses, sore deep tissues, phobia to needles and any allergy were excluded from the
153 study.
154
155 ***Experimental design***
156 In a two-way repeated-measures experimental design, participants performed an isometric
157 torque matching and reproduction task with either pain (a single intramuscular injection of
158 hypertonic saline) or a placebo control (a single intramuscular injection of isotonic saline)
159 (condition factor). Participants attended a familiarisation session, and then completed the
160 experimental conditions in a randomised and counterbalanced order, with each visit separated
161 by a minimum of seven days. During the task participants attempted to produce torque at two
162 set targets without the aid of real-time visual feedback before (Baseline), during (Pain/No
163 Pain), and after (Recovery) the induction of pain and no pain (time factor). Measures of
164 torque, rating of perceived effort (RPE), surface electromyography (sEMG) and heart rate
165 (HR) were taken during each contraction. Pain intensity was recorded continuously using an
166 electronic visual analogue scale (VAS) and pain quality through the completion of a McGill
167 Pain Questionnaire (MPQ). A schematic of the experimental design and protocol is outlined
168 in Figure 1.
169
170 <FIG 1 HERE>
171
172 ***Experimental Procedures***
173 *Torque matching and reproduction task*
174 All visits were performed seated on an isokinetic dynamometer (Cybex HUMAC Norm
175 isokinetic dynamometer; CSMi, Soughton, MA, USA) set up for the right leg, with the knee

176 set at an angle of 75^{o} of flexion (0^{o} = full extension of the knee), and a hip angle of 90^{o}.
177 Torque matching and reproduction for knee extension were determined at isometric
178 contractions of 15% and 20% maximal voluntary isometric torque (MVIT). These values
179 were selected based on the percentage of MVIT utilised during maximal (100% maximal
180 oxygen uptake; VO_{2MAX}) and submaximal (70% VO_{2MAX}) cycling exercise performed at a
181 pedal rate between 60-80 revolutions per minute (24). At the start of each visit, participants
182 completed 3×3 s maximum voluntary isometric contractions (MVICs) separated by 90 s rest,
183 with the greatest instantaneous value taken as MVIT. If the MVIT of consecutive MVICs
184 were not within 5% of each other, additional MVICs were performed until this criteria was
185 achieved.
186
187 Participants attempted the target torques in a trial with real-time torque-production visual
188 feedback ('Feedback Trial') and a trial without visual feedback ('No Feedback Trial').
189 During the Feedback Trials, target torques (15% and 20% MVIT) were presented with actual
190 torque produced via a computer display. Participants were instructed to remember muscular
191 sensations experienced during each target torque and use these to reproduce the same torque
192 in the subsequent No Feedback Trial (7). All Feedback and No Feedback trials were
193 separated by a 3-minute period of rest.
194
195 For each trial, participants performed four 6 s contractions separated by 4 s of rest in a
196 randomised counter-balanced order, which provided two attempts at both target torques (i.e.
197 2×15% MVIT, 2×20% MVIT). During each contraction, participants were instructed to try
198 and match the target torque within the first 2 s, and then maintain it for a further 4 s.
199
200 *Intramuscular injection procedure*

A single bolus of 1.0 mL hypertonic saline (5.8%) was manually injected into the middle third of the VL of the right leg over a 20 s window (10 s infusion period). The injection was performed using a 3 mL Luer-Lok syringe connected to a 25 G × 38 mm SurGuard2 disposable stainless needle (Terumo, Japan). In the control condition, a single bolus of 1.0 mL isotonic saline (0.9%) was injected.

Visit 1 – Familiarisation

Participant anthropometric and descriptive measures of age, height, body mass, and hours of physical activity engaged in per week were recorded. Participants were then familiarised with the RPE and pain scales (8), as well as the performance of MVICs, and the Feedback/No Feedback Trials. Five minutes after the completion of the final MVIC, participants performed an initial Feedback Trial followed by a No Feedback Trial. Verbal confirmation of the actual torque produced in each contraction was given after the completion of the trial. All four contractions in the No Feedback Trial were required to be within 10% of target torque, with further No Feedback Trials completed until this was satisfied. The visit concluded upon the successful completion of a No Feedback Trial or following ten unsuccessful trials.

Visits 2 & 3 – Experimental visits

All participants completed a Control (isotonic saline) and an Experimental (hypertonic saline) condition in a randomised and counterbalanced order. In each condition, five-minutes after the completion of the MVICs, participants completed six trials (Feedback, No Feedback, Feedback, No Feedback, Feedback, No Feedback). Prior to the second No Feedback Trial, participants received an intramuscular injection of either isotonic (Control) or hypertonic saline (Experimental), with the No Feedback Trial beginning 20 s after the removal of the needle. This ensured that the 15% and 20% MVIT contractions in this No Feedback Trial

were performed with a “moderate” to “strong” muscle pain intensity elicited from the painful hypertonic saline infusion. Ten minutes after the completion of this second No Feedback Trial, the final Feedback and No Feedback (Recovery) Trials were performed.

Perceptual and psychological measurements

At the start of each visit participants rated the expected pain (0 = “no pain” to 10 = “worst possible pain”) and their confidence to cope with it (0 = “not confident at all” to 10 = “completely confident”). Muscle pain was evaluated by intensity and quality. Participants rated pain intensity on a moment-to-moment basis using an electronic VAS ranging from 0 (“no pain”) to 10 (“extremely intense pain”). Participants were instructed to anchor the scale to previous experiences of EIP (4). The device recorded the reported pain value every 5 s, providing measures of pain for each individual contraction. In addition, onset pain intensity (VAS onset), maximal pain intensity (VAS peak), time to maximal intensity (VAS time to peak; from the commencement of sampling), mean pain intensity (VAS mean) and duration of pain (VAS duration; from VAS onset until the state of “no pain”) were also calculated using data from the electronic VAS.

After the second No Feedback Trial, when pain had subsided, Total Pain Rating Index and Subclass Rating Index was calculated using a 78 item MPQ (29), with overall quality of pain described by descriptors (sensory, affective, evaluative and miscellaneous) chosen by more than one-third of participants. Upon the completion of each trial, participants provided a RPE, defined as the effort to drive the limb (32), of both target torques using the 15-point Borg (6-20) scale (6).

Physiological measurements

Heart rate (HR) was recorded upon the completion of each individual contraction, and muscle electrical activity was continuously recorded using surface electromyography (sEMG). sEMG was attained through square surface electrodes (Ag/AgCl, 32 × 32 mm; Nessler Medizintechnik, Innsbruck, Austria) mounted in a bipolar set-up, and placed on the muscle belly of the VL, vastus medialis (VM) and rectus femoris (RF). For each muscle a reference electrode was placed on the patella. Prior to application of the electrodes, the skin was shaven and cleansed with an alcohol swab. The electrical signal was sampled at 1000 Hz (Biopac MP150, Biopac Systems Inc., California, USA).

Data analysis

The sEMG and torque data (for analysis of torque output complexity) were analysed using custom code written in MATLAB 2018a (The MathWorks, Massachusetts, USA).

Torque and error

Torque was recorded through Spike2 software (Cambridge Electronics Design (CED), Cambridge, UK). For each 6 s contraction, the torque produced over the last 4 s was averaged. The average of the actual torque produced for each 15% and 20% target was used to define the error in participant torque reproduction. Error was defined as the unidirectional difference between the required target torque and the actual torque produced, and expressed as a percentage of MVIT (i.e. actual torque of 17.5% MVIT for the 15% MVIT target would be equal to an error of 2.5% MVIT). All values of error are presented as positive integers regardless of whether the participant over- or undershot the target torque. The pain on the VAS reported for the corresponding contractions were also averaged for the two attempts at each target torque to provide a mean VAS value for each target torque.

Surface electromyography (sEMG)

To create a linear envelope representation of the data, rectified absolute values of the raw sEMG signals were two-pass zero-lag filtered using a fourth-order low-pass Butterworth filter, with a cut-off frequency of 5 Hz. The amplitude for the VL, RF and VM were averaged over the final 4 s period of each 6 s contraction. These values were normalised to the maximum amplitude of the prior MVICs (% MVIC). For each trial, the sEMG activity was averaged for the two contractions performed at each target torque.

Torque complexity

The complexity and regularity of the torque output was estimated through the use of approximate entropy (ApEn) and sample entropy (SampEn) (37, 43). When applied to physiological time-series data, ApEn is an index that quantifies the predictability or probability of the subsequent values based on prior values, whilst SampEn provides the same output but excludes self-matches (37, 43). Both ApEn and SampEn are defined by a value between 0 ('high regularity, low complexity') and 2 ('low regularity, high complexity'). A detailed guide to the algorithms for the calculation of ApEn are evidenced in the appendix of Slifkin and Newell (48), whilst SampEn was calculated using the parameters outlined by Pethick and colleagues (34).

Statistical analysis

To compare reproduction error between the Control and Experimental conditions at the three time-points (Baseline, Pain/No Pain, and Recovery), a Levene's test was used to determine equality of variance for each normalised target torque (15% and 20% MVIT). Changes in HR, RPE, sEMG activity and complexity were evaluated using two-way Analysis of variance (ANOVA) with treatment factor with two fixed levels (Control, Experimental) and a repeated

measures Time factor with two time-points (Baseline, Pain/No Pain). A two-way ANOVA with a treatment factor with two fixed levels (No Feedback, Feedback) and a repeated measures Time factor with two time-points (Baseline, Pain/No Pain) was also implemented to evaluate changes in complexity. When an interaction effect was observed, follow-up paired samples t-tests were used to assess differences between conditions. Paired samples t-tests were also implemented to evaluate the differences between conditions for pain expectation and confidence, VAS scores, pre-test PANAS, and the change in torque produced in Baseline compared to the Pain/No Pain time-point. A Pearson Bivariate correlation was used to evaluate the correlation between torque error and VAS score reported during the Pain/No Pain contractions. Cohen's guidelines of 0.1 (small), 0.3 (medium) and greater than or equal to 0.5 (large) were used to indicate the strength of correlation.

All data was checked for the standard assumptions associated with the performance of the above statistical tests prior to analysis. Data that did not satisfy the Shapiro-Wilk test of normality ($P<0.05$) were logarithmically transformed. Results are presented as mean ± standard deviation (SD). Cohen's *d* and partial eta square (η_p^2) values are reported as measures of effect size. Statistical significance was accepted at an alpha level of $P<0.05$. All statistical analysis were completed using SPSS Statistics v25.0 (SPSS, IBM, New York, USA).

RESULTS

Experimental muscle pain

As shown in Table 1, paired samples t-tests revealed a significant difference in VAS pain data between the Control and Experimental conditions. The pain experienced in Experimental was significantly greater in terms of the onset VAS pain reported, with a significantly longer

time to peak, yet greater peak VAS pain compared to Control. The reported VAS pain in Experimental was also longer in duration, inducing a significantly greater mean VAS pain, equivalent to a "moderate" to "somewhat strong" muscle pain, and therefore producing a greater overall VAS pain area than Control.

The pain experienced in Experimental was predominantly described in the sensory and evaluative dimensions of pain as "aching" (50% of participants), "throbbing" (43% of participants), "shooting" (36% of participants), "cramping" (36% of participants), "annoying" (36% of participants). This produced a mean Total Pain Index of 14 ± 8, with an overall Present Pain Intensity of 2.1 ± 0.7 ("discomforting").

During the Pain/No Pain trial, a paired samples t-test revealed no significant difference (t_{13}=-0.9, P=0.366, $CI_{.95}$ -0.9, 0.3, d=0.1) in mean VAS between contractions performed at 15% MVIT (4.2 ± 1.9) and 20% MVIT (4.5 ± 2.2) in the Experimental condition. Each of the two target torques in the Pain/No Pain trial was therefore completed at a similar intensity of pain (Fig 2b. and Fig 3b.).

<FIG 2 HERE>

<FIG 3 HERE>

Paired samples t tests revealed no significant difference (t_{13}=-1.8, P=0.096, $CI_{.95}$ -2.08, 0.19, d=0.5) in expectations of pain between the Control (4.5 ± 2.1) and Experimental (5.4 ± 1.8) conditions, with no significant differences in the confidence to cope with the expected pain

(t_{13}=0.2, P=0.818, $CI_{.95}$ -0.29, 0.37, d=0.1) between Control (9.5 ± 1.0) and Experimental (9.4 ± 1.0).

Comparisons of torque production accuracy

In the presence of greater levels of pain, participants demonstrated an increased variability in their ability to reproduce target torque without visual feedback. However, once the pain had subsided, participants were able to produce the target torque with the same accuracy as Baseline. This is demonstrated by the Levene test for equality of variance, which revealed a significant difference in the variance of mean contraction torque in the Pain/No Pain trial between the Experimental and Control conditions at both 15% MVIT ($F_{1,26}$=4.3, P=0.049, d=0.6) and 20% MVIT ($F_{1,26}$=12.0, P=0.002, d=1.0), as shown in Figures 4 and 5. There was no correlation between Pain/No Pain error and the pain intensity reported during the contractions (15% MVIT; r= -0.053, P=0.858, 20% MVIT; r=0.172, P=0.557). In addition, there was no significant difference in variance between conditions at the Baseline (15% MVIT; $F_{1,26}$=0.2, P=0.612, d=0.1, 20% MVIT; $F_{1,26}$=2.1, P=0.161, d=0.2) and Recovery (15% MVIT; $F_{1,26}$=1.8, P=0.195, d=0.2, 20% MVIT; $F_{1,26}$=3.9, P=0.058, d=0.4) time-points.

<FIG 4 HERE>

<FIG 5 HERE>

A paired samples t-test found no significant difference in the change in torque mismatch between Baseline and Pain/No Pain trials at 15% MVIT (t_{13}=-1.5, P=0.169, $CI_{.95}$ -1.1, 0.2, d=0.5) when comparing the Control (2.5 ± 1.7 %MVIT) and Experimental (4.8 ± 4.8 %MVIT) conditions. Furthermore, the paired samples t-test highlighted no significant

375 difference in the same change in torque mismatch between Control (4.2 ± 3.5 %MVIT) and
376 Experimental (7.4 ± 6.0 %MVIT) when contractions were performed at 20% MVIT (t_{13}=-1.3,
377 P=0.235, $CI_{.95}$ -1.6, 0.4, d=0.4). This suggests that the target torque absolute error in the
378 'Pain/No Pain' was similar to the error made at Baseline despite the change in pain
379 experienced.
380
381 *Rating of perceived effort*
382 It was apparent that the effort experienced during the contraction was greater in the presence
383 of increased pain, when performed at 20% MVIT. The 2 x 2 (condition x trial) repeated
384 measures ANOVA demonstrated a significant interaction effect at 20% MVIT for RPE over
385 trials between conditions ($F_{1,13=}$6.0, P=0.030, η_p^2=0.314). Follow-up paired samples t-tests
386 revealed a significantly greater RPE (t_{13}=-2.3, P=0.038, $CI_{.95}$ -1.31, -0.04, d=0.3) in the
387 Pain/No Pain trial in Experimental compared to Control. A significantly greater (t_{13}=-2.4,
388 P=0.033, $CI_{.95}$ 0.1, 1.8, d=0.4) RPE was also reported in the Experimental condition at the
389 Pain/No Pain trial compared to the Baseline trial. No significant main effect of condition was
390 observed at either 15 or 20% MVIT (P>0.05). A significant effect of trial was reported at
391 20% MVIT ($F_{1,13=}$5.2, P=0.041, η_p^2=0.284), but not at 15% MVIT (P>0.05) (Figs. 2c., 2d.,
392 3c. and 3d.). There was no interaction effect observed at 15% MVIT (P>0.05).
393
394 *Surface electromyography (sEMG)*
395 Due to excessive noise in sEMG signal, two participants were removed from the dataset and
396 analysis was performed on the remaining participants (n=12). Despite a greater variance in
397 mean contraction torque in the presence of muscle pain, there were no discernible alterations
398 in activation of the agonist and synergist muscles. At 15 and 20% MVIT, the performance of
399 a 2 x 2 (condition x trial) repeated measures ANOVA demonstrated no significant main effect

400 of condition or trial in either the VL, VM or RF (P>0.05). The VL, VM or RF also
401 demonstrated no significant interaction effect for sEMG activity over trial between conditions
402 at both target torques (P>0.05).
403
404 *Torque complexity*
405 As shown in Table 2, the presence of visual feedback resulted in a more complex (less
406 regular) torque signal (assessed by both ApEn and SampEn) than when torque was being
407 reproduced (No Feedback Trials) (P<0.001). No condition (P>0.05) and no interaction
408 effect was observed for either ApEn or SampEn (P>0.05) at both target torques. At 15 and
409 20% MVIT, the performance of a 2 x 2 (condition x trial) repeated measures ANOVA
410 demonstrated no significant main effect of condition for either ApEn or SampEn, as well as
411 no significant main effect of trial for either complexity statistic (P>0.05). There was no
412 interaction effect observed for either ApEn or SampEn (P>0.05) at both target torques.
413
414 *Heart rate (HR)*
415 The 2 x 2 (condition x trial) repeated measures ANOVA revealed no significant main effect
416 of condition at 15 or 20% MVIT (P>0.05). At 15% MVIT there was no significant main
417 effect of trial (P>0.05), however there was at 20% MVIT ($F_{1,13}$=5.2, P=0.041, η_p^2=0.284). No
418 significant interaction effect for HR and trial between conditions was observed at 15 or 20%
419 MVIT (P>0.05).
420
421 **DISCUSSION**
422 The present study demonstrates for the first time that the experience of muscle pain,
423 administered by the intramuscular injection of hypertonic saline into the VL, resulted in a
424 greater variance in the mean contraction torque at both 15 and 20% MVIT when compared to

425 the injection of isotonic saline (a placebo control). The increased variance was paralleled by
426 an elevated experience of pain at both contraction intensities, and a greater perceived effort
427 when performed at 20% MVIT. Once the pain had subsided, accuracy of torque production
428 returned to baseline levels. This study for the first time demonstrates that the presence of
429 muscle pain (that feels like EIP) impedes the ability to accurately reproduce torque in the
430 knee extensors. This important finding provides key experimental evidence for the
431 deleterious implications of EIP on the ability to self-regulate exercise intensity.
432
433 *Effect of pain on isometric torque reproduction*
434
435 The purpose of the present study was to establish whether the presence of pain in a muscle
436 with a major contributing role to force generation during both dynamic contractions and
437 whole-body exercise (i.e. the VL) has a debilitative effect on producing a given torque using
438 the ipsilateral knee extensor muscle group. The primary finding from this study is that the
439 mismatch between the actual torque produced and the target torque (when required to
440 reproduce both 15 and 20% MVIT) was significantly more variable with pain, with no
441 discernible direction of error (i.e. participants both under- and overshot the target torque).
442 Resultantly, this study is the first to demonstrate that the experimental induction of pain in a
443 large locomotor muscle group impairs the judgement of torque during an isometric
444 reproduction task performed at an intensity of relevance to endurance exercise performance.
445
446 The compromised ability to accurately reproduce torque during pain is in line with previous
447 research that has implemented the hypertonic saline model in the elbow flexors to investigate
448 the impact of pain on estimation error in a contralateral torque estimation task (40, 41, 57).
449 However, this prior literature has consistently reported that participants specifically

450 *overestimated* the torque that is produced in the painful muscle, and therefore produced less
451 torque than required. In contrast with lack of direction in error reported in the present study,
452 this observed disparity could be due to potential differences in the limb evaluated (e.g.
453 contralateral or ipsilateral). Alternatively, as the knee extensor muscles respond differently to
454 exercise-induced fatigue (55), the muscle group tested (elbow flexor vs. knee extensors)
455 should also be considered.
456
457 *Proposed mechanisms*
458 The presence of the hypertonic saline solution in addition to the short-duration muscle
459 contraction creates a noxious environment within the skeletal musculature (31), which results
460 in an alteration in activity of both ascending metaborecptive and nociceptive group III and IV
461 afferent fibers (18). In this noxious environment, there are several neuromuscular
462 mechanisms that, when acting in singularity or in combination, may provide an explanation
463 for the impaired reproduction of torque in the present study.
464
465 Convergent projection from group III and IV afferents on common interneurons from group
466 Ib proprioceptive afferents (45) provide information on muscle force (15). As discussed by
467 Salomoni and Graven-Nielsen (44), the large variance in the mean contraction torque in the
468 Experimental condition could be a result of the spatial facilitation between these afferents
469 interfering in the central interpretation of proprioceptive information essential for the
470 accurate control of torque. A discrepancy between the centrally mediated judgement of
471 torque and the actual afferent feedback from the periphery could therefore have resulted in
472 the torque reproduction error.
473

In addition, the projection of the group III and IV afferents have inhibitory effects on the central nervous system. The increased afferent feedback from the hypertonic saline may have limited motor cortical excitability, and reduced central motor drive and voluntary activation of the knee extensors (14, 19). In order to compensate for the hypertonic saline-induced impairment of motor cortex excitability, a greater effort is required to drive the limb to meet the required torque (30, 39). As an outcome reflected in the present study, this could provide a possible explanation for some of the differences in actual and perceived torque produced. The findings from Proske and colleagues (40) where the matching of torque through effort resulted in an overshoot of the target torque, are in support of this explanation.

Despite the observed impairment in torque-reproduction performance during pain, there was no change in the torque complexity of the knee extensors, or the level of muscle activity assessed by sEMG. The absence of alterations in sEMG is comparable with findings from the established literature into the implications of EIP on muscle activity during submaximal isometric contractions, where a lack of marked changes in sEMG signal are also observed (16, 44, 46). Combined, these observations contradict the underpinning theory of the 'Pain Adaptation Model' (25) where it is predicted that the presence of pain has a reliable inhibitory influence on agonist muscles, whilst simultaneously activating the antagonists. Instead, the observations of the present study could, with caution, be in-line with the "moving differently in pain" model proposed by Hodges and Tucker (17). This theory postulates that pain initiates a non-uniform effect across the motor neurone pool, causing a redistribution of activity between and within muscles to provide a key adaptive and protective function. Whilst this alteration has the immediate benefit of minimising the pain experienced and preventing further injury or damage to the area in pain during muscular contraction, this change to a "sub-optimal" movement strategy could have consequences for the efficiency of task

performance (17, 53). Detection of these adaptations would however require the use of fine-wire electrodes (52) or high density sEMG, as a combination of changes in order of motor unit activation or synchronisation can occur without alteration in amplitude of gross sEMG (51).

A loss of knee-extensor torque complexity during both prolonged maximal and submaximal contractions has been closely associated with fatigue (34, 35), and is suggested to have a detrimental impact on the performance of motor tasks in the lower limb (10). In the present study, the lack of change in torque complexity suggests that the acute pain from the hypertonic saline was unlikely to have independently caused neuromuscular fatigue. The increased variance in mean contraction torque is therefore unable to be explained by pain-induced mechanisms of fatigue during the short-duration and submaximal isometric contractions.

This finding is consistent with prior literature, where differences in torque complexity are not observed in the first few seconds of isometric muscle contraction despite the presence of pain (from an eccentric contraction muscle damage protocol) and the consequential impaired ability to perform a maximal voluntary contraction (33). As torque complexity progressively decreases over time during submaximal contractions until the point of task failure (34), if the torque reproduction task in the present study was performed over a longer duration, a pain-induced *acceleration* of exercise-induced fatigue (and therefore loss of torque complexity) would likely be observed in addition to the impaired the ability to accurately reproduce torque. As such the findings of the present study reinforce the notion that acute, moderate muscle pain alone is not necessarily fatiguing, but may accelerate the development of fatigue during prolonged or exhaustive exercise (27, 50), or impair maximal voluntary contraction.

A further point of consideration is that in the absence of visual feedback, and sole reliance on afferent/efferent information and task memory, the ability to accurately reproduce torque depreciates (22) and that this is characteristically coupled with a lower complexity of the torque signal (indicative of a reduced adaptability in force control) (21, 49). This observation is replicated in the present study, and it is noteworthy that the values for ApEn and SampEn in the No Feedback conditions are similar to those shown at task failure in exhaustive exercise (34). Therefore, it is possible that the induction of muscle pain in the present study was not able to reduce the complexity of the torque signal beyond that already caused by the removal of visual feedback.

Alternatively, the compromised ability to accurately reproduce torque (despite no change in loss of torque complexity) could be due to the experience of pain preventing some attentional focus on the task (23), making the task more challenging. It is plausible that the elevated intensity of pain (induced by the injection of hypertonic saline), which was rated as "moderate" to "somewhat strong" in both target torques, provided a stimulus which was perceived as threatening. With some attentional resources focused on coping with the 'threat' of the noxious stimuli, attention may have been directed away from the task, which could have resulted in a compromised accuracy of torque reproduction (11); a notion supported by evidence from previous experimental work (5, 26). However, in the current study, there was no relationship between pain intensity and error, which indicates that the sensation of pain alone was unlikely to have had a direct influence on task performance.

Overall, it is evident that the presence of pain interferes with proprioception during submaximal isometric contractions in the lower-limb. The design and findings of the present

549 study therefore provide a key indication of the potential mechanism underpinning the
550 detrimental effect of EIP on exercise intensity regulation and endurance performance. Some
551 caution should however be taken when extrapolating these findings to whole-body exercise.
552 In order to improve task relevance to whole-body locomotor exercise and further apply the
553 findings of the present study, there is the need for the impact of this experimental model to be
554 evaluated during isokinetic or dynamic muscular contractions performed at a varying or
555 higher work rate.
556
557 *Methodological considerations*
558 Whilst there is inconsistent evidence for sex-related differences in the pain intensity response
559 to the hypertonic saline model (20, 42), the fluctuations in hormone concentration across the
560 different menstrual cycle phases may cause differences in pain perception to experimental
561 pain (47). It is acknowledged that the present study did not account for menstrual cycle
562 phases of the female participant, and this is a limitation. It is also important to note that the
563 short-duration and submaximal isometric contractions used in the current study were not
564 fatiguing, and this limits the ability to examine the notion that pain accelerates the
565 development of exercise-induced fatigue in addition to the impairment in accurate torque
566 reproduction. To explore this in combination, future investigations should attempt to employ
567 a similar study design examining torque reproduction ability in the presence of muscle pain
568 during contractions performed at a greater exercise intensity, or over a longer duration.
569
570 *Conclusion*
571 In conclusion, the injection of hypertonic saline into the VL during a torque reproduction task
572 created muscle pain that resulted in an impaired ability to accurately produce a given
573 submaximal target torque during a short, submaximal isometric contractions. The presence of

pain was linked with a greater effort to drive the limb and meet the given target torque when attempting to contract at 20% MVIT, but not at 15% MVIT. The compromised ability to reproduce torque returned to baseline levels once pain had subsided. These findings have implications for the impact of EIP on self-selected work rate regulation during endurance exercise performance.

599

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774 **FIGURE CAPTIONS**

775 **Fig 1.** Schematic overview of the experimental design and procedures. MVICs: maximal
776 voluntary contractions

777

778 **Fig 2.** Individual (*open symbol*) and group mean (*filled symbol*) perceptual differences
779 between conditions (Control and Experimental) at Baseline, Pain/No Pain and Recovery
780 time-points at a target torque of 15% MVIT. Differences in pain intensity after injection of
781 isotonic saline (Control, *a*) and hypertonic saline (Experimental, *b*). Differences in RPE in
782 Control (*c*) and Experimental (*d*) conditions. *Significantly greater where hypertonic saline
783 was injected

784

785 **Fig 3.** Individual (*open symbol*) and group mean (*filled symbol*) perceptual differences
786 between conditions (Control and Experimental) at Baseline, Pain/No Pain and Recovery
787 time-points at a target torque of 20% MVIT. Differences in pain intensity after injection of
788 isotonic saline (Control, *a*) and hypertonic saline (Experimental, *b*). Differences in RPE in
789 Control (*c*) and Experimental (*d*) conditions. *Significantly greater where hypertonic saline
790 was injected

791

792 **Fig 4.** Individual (*open circle*) and group mean (*filled circle*) torque reproduction error at a
793 target torque of 15% MVIT before (Baseline), during (Pain/No Pain) and after (Recovery)
794 injection of isotonic saline (Control, *a*) or hypertonic saline (Experimental, *b*).

795

796 **Fig 5.** Individual (*open circle*) and group mean (*filled circle*) torque reproduction error at a
797 target torque of 20% MVIT before (Baseline), during (Pain/No Pain) and after (Recovery)
798 injection of isotonic saline (Control, *a*) or hypertonic saline (Experimental, *b*).

ADDITIONAL INFORMATION

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Disclosures

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1. Summary VAS pain data across the entire duration of the Control and Experimental conditions

	Control	Experimental	*P*
VAS mean	0.8 ± 1.0	3.1 ± 1.0**	<0.001
VAS peak	1.6 ± 2.2	5.7 ± 1.7**	<0.001
VAS onset	0.5 ± 0.8	1.7 ± 1.3*	0.012
VAS time to peak (s)	41 ± 29	71 ± 24*	0.020
VAS duration (s)	55 ± 56	233 ± 60**	<0.001
VAS area	86.3 ± 115.4	759.8 ± 325.6**	<0.001

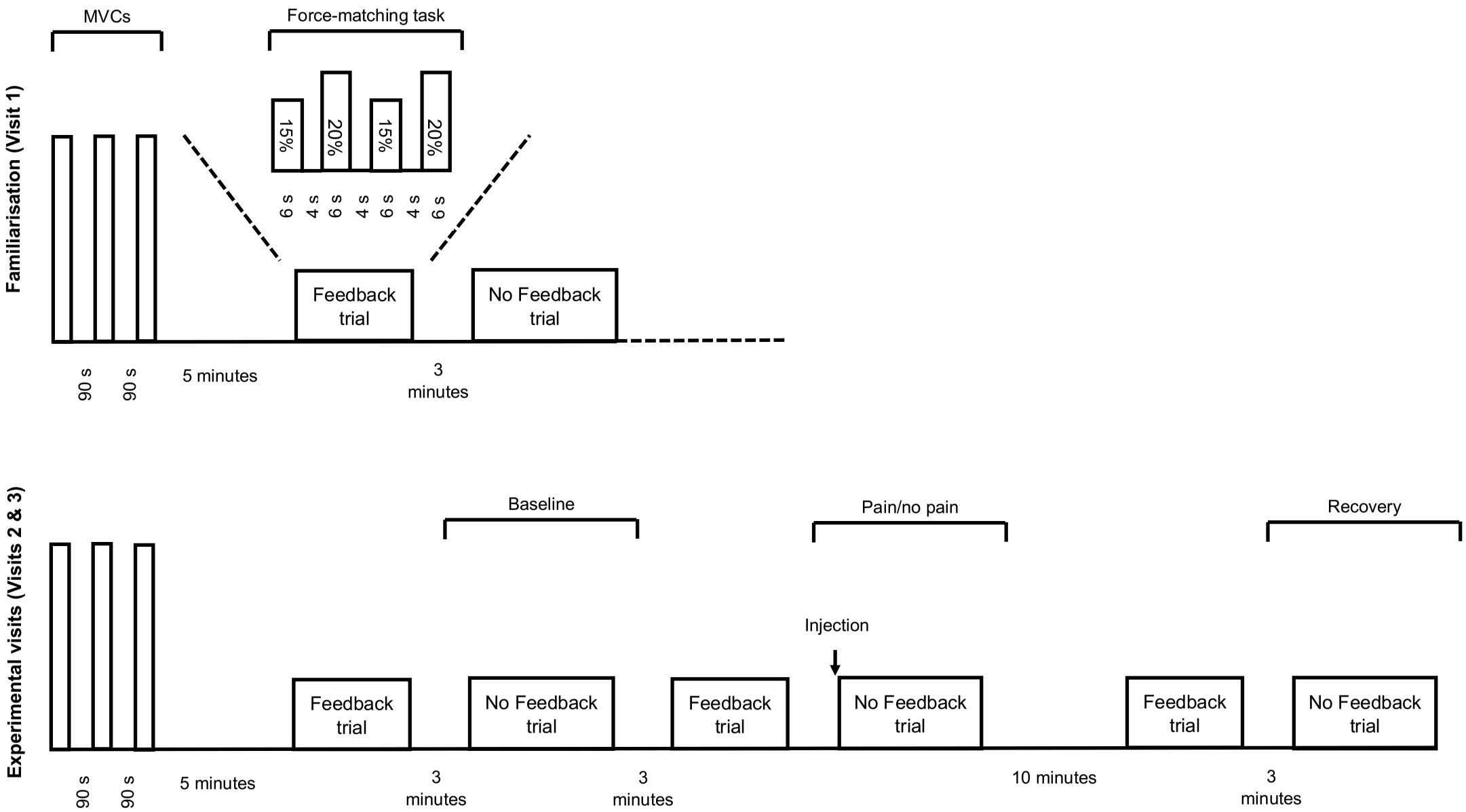
Values are means ± SD. **Significant difference between Control and Experimental ($P < 0.001$). *Significant difference between Control and Experimental ($P < 0.05$). VAS scale 0 (no pain) to 10 (extremely intense pain)

Table 2. Torque complexity (ApEn) during Feedback and No Feedback trials at the Baseline and Pain/No Pain time-points

Condition	Time-point	Trial	Target Torque			
			15% MVIT		20% MVIT	
			ApEn	SampEn	ApEn	SampEn
Control	Baseline	Feedback	0.71 ± 0.25*	0.71 ± 0.29*	0.57 ± 0.22*	0.56 ± 0.27*
		No Feedback	0.35 ± 0.17 *	0.32 ± 0.17*	0.31 ± 0.21*	0.29 ± 0.22*
	Pain/No Pain	Feedback	0.73 ± 0.21*	0.72 ± 0.24*	0.60 ± 0.26*	0.61 ± 0.30*
		No Feedback	0.35 ± 0.21*	0.32 ± 0.22*	0.28 ± 0.17*	0.26 ± 0.17*
Experimental	Baseline	Feedback	0.78 ± 0.24*	0.79 ± 0.30*	0.64 ± 0.21*	0.64 ± 0.25*
		No Feedback	0.29 ± 0.13*	0.26 ± 0.13*	0.27 ± 0.12*	0.24 ± 0.12*
	Pain/No	Feedback	0.74 ±	0.75 ±	0.68 ±	0.68 ±

Pain		0.27*	0.31*	0.23*	0.28*
	No Feedback	0.32 ± 0.19*	0.29 ± 0.19*	0.22 ± 0.11*	0.20 ± 0.10*

Values are means ± SD. * Significant difference between Feedback and No Feedback trial within condition and time-point ($P < 0.001$).


Familiarisation (Visit 1)
MVCs
Force-matching task
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20%
15%
20%
6 s
4 s
6 s
4 s
6 s
4 s
6 s
Feedback trial
No Feedback trial
90 s
90 s
5 minutes
3 minutes
Experimental visits (Visits 2 & 3)
Baseline
Pain/no pain
Recovery
Injection
Feedback trial
No Feedback trial
Feedback trial
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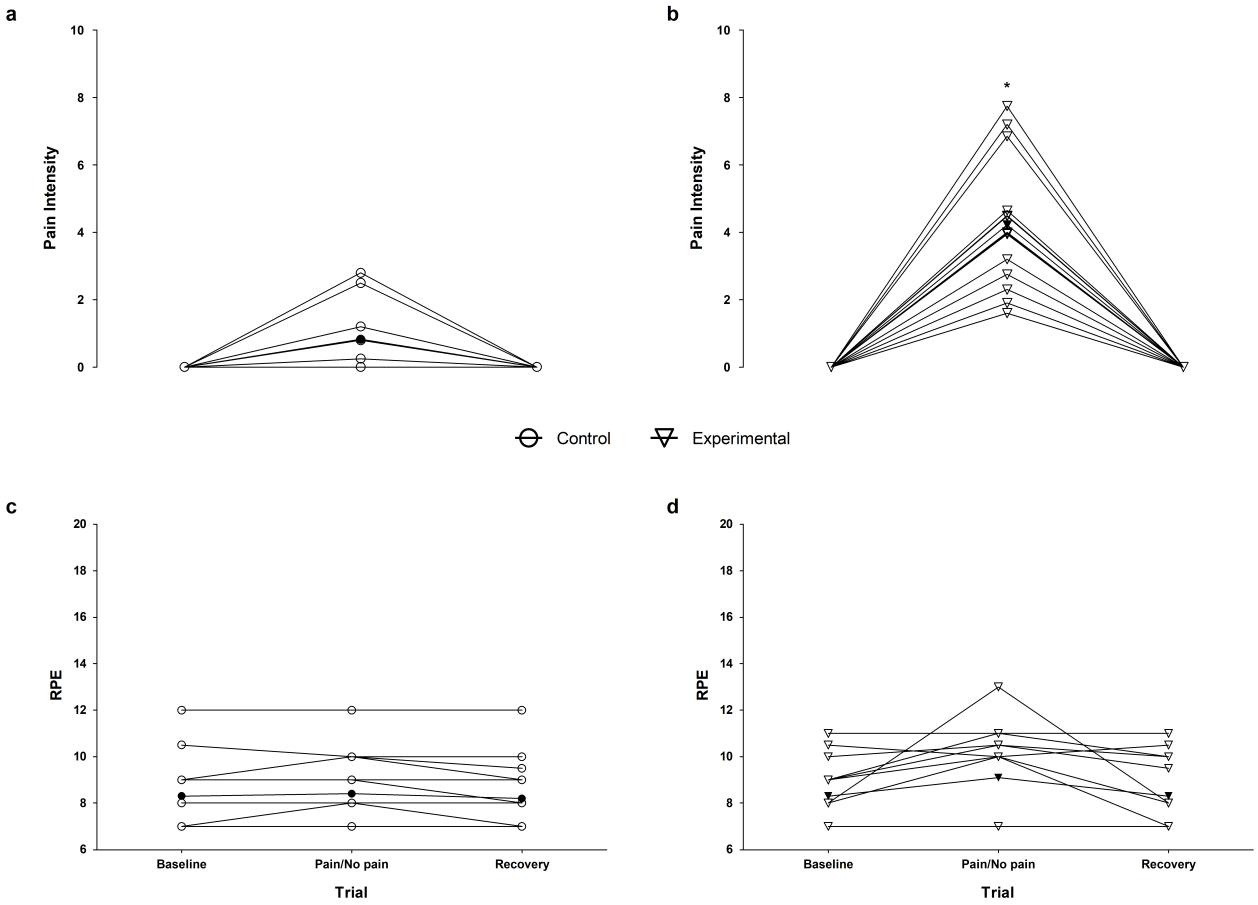
Feedback trial
No Feedback trial
90 s
90 s
5 minutes
3 minutes
3 minutes
10 minutes
3 minutes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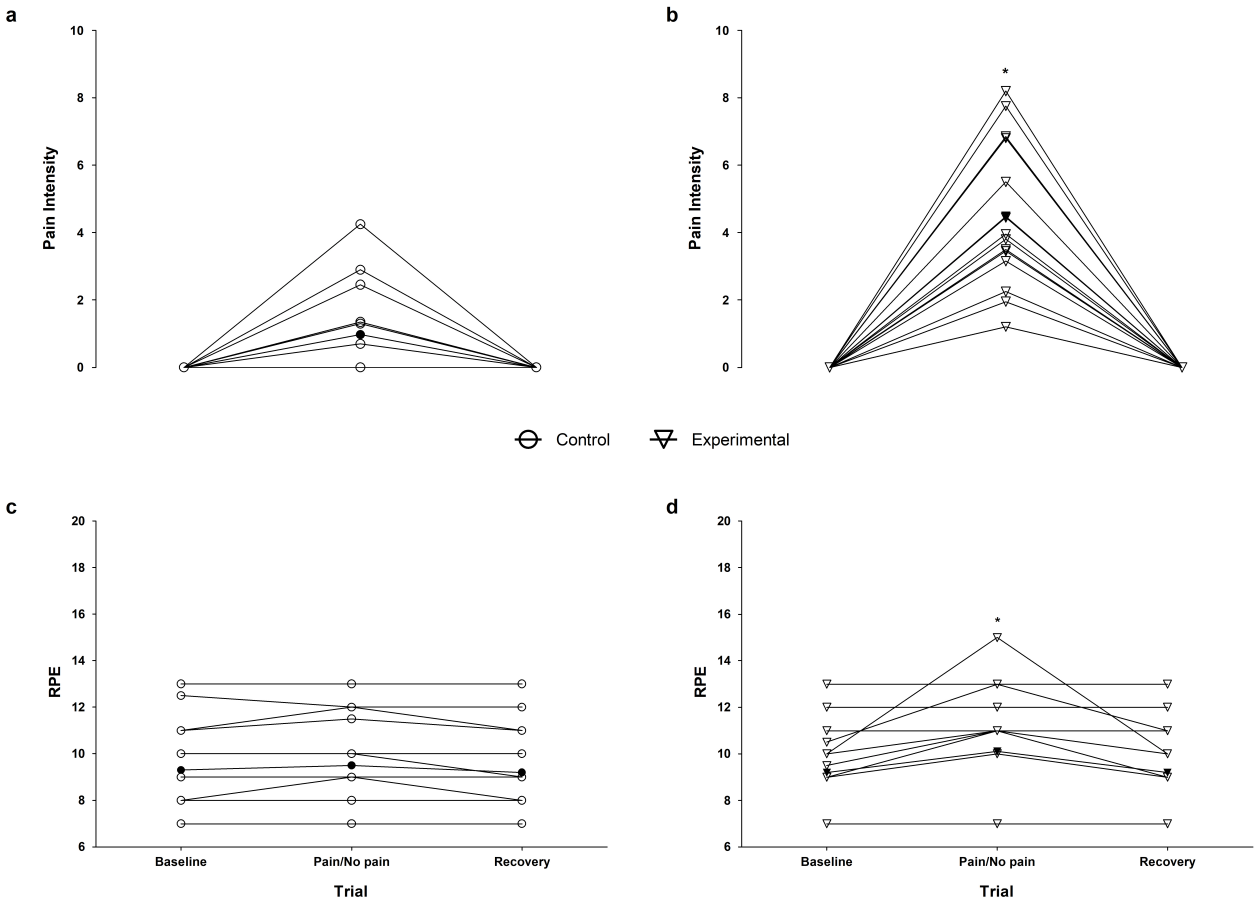
Recovery
Trial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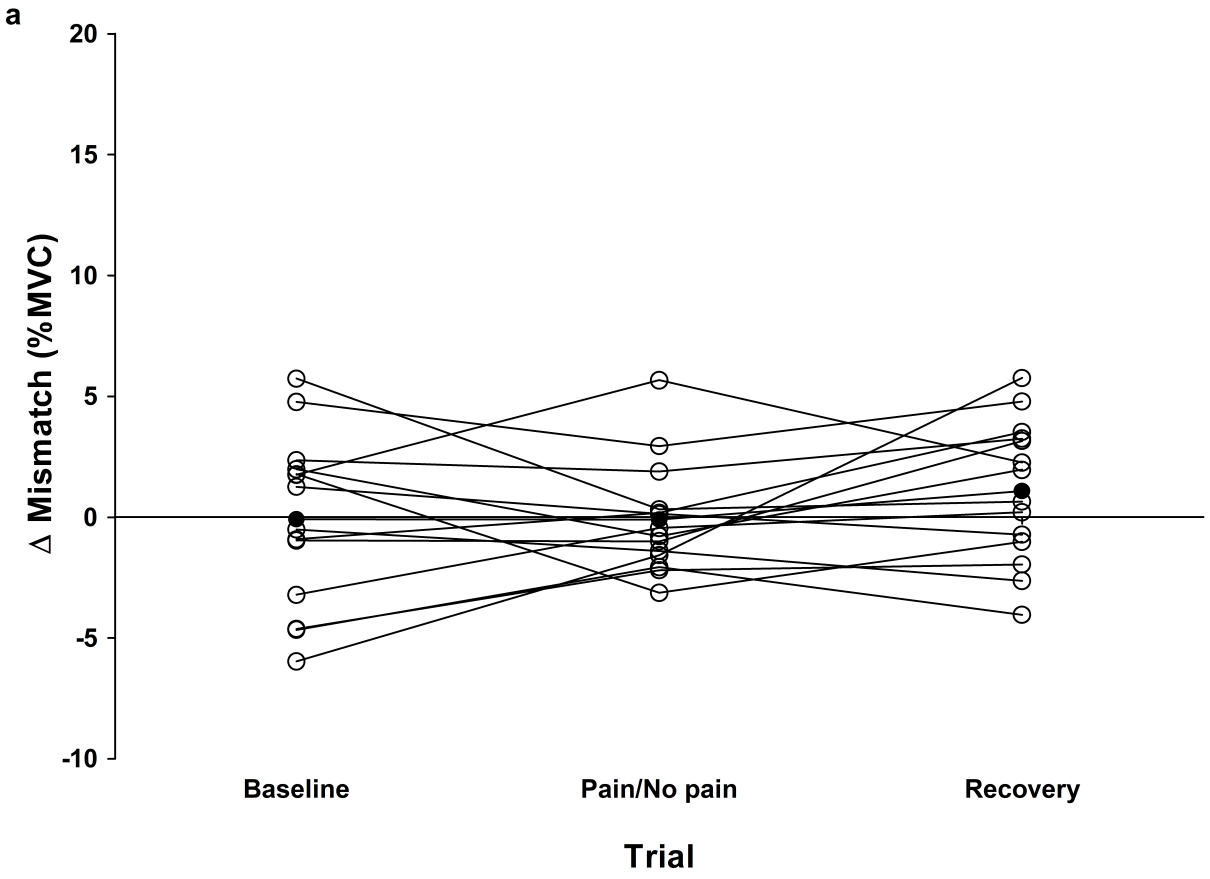
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Δ Mismatch (%MVC)
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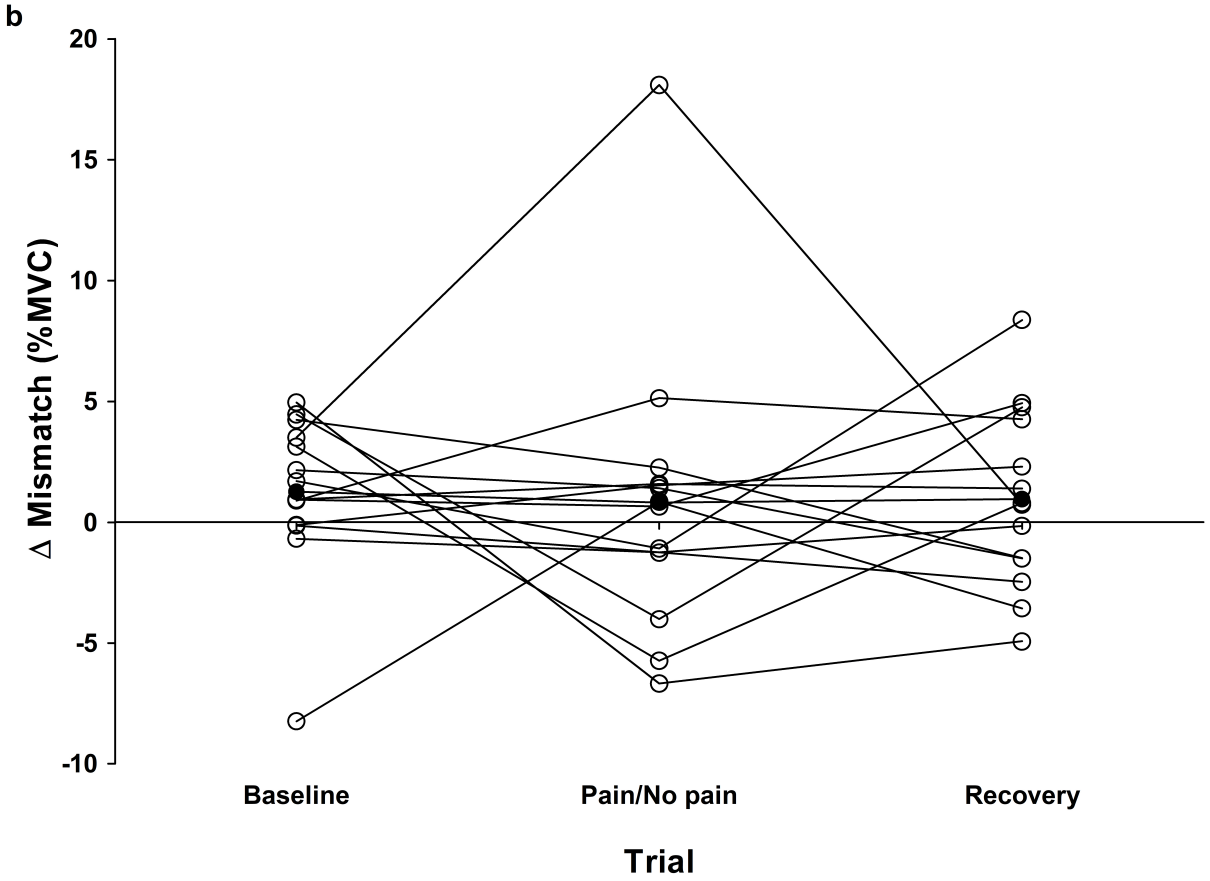
Pain/No pain
Recovery
Trial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Δ Mismatch (%MVC)
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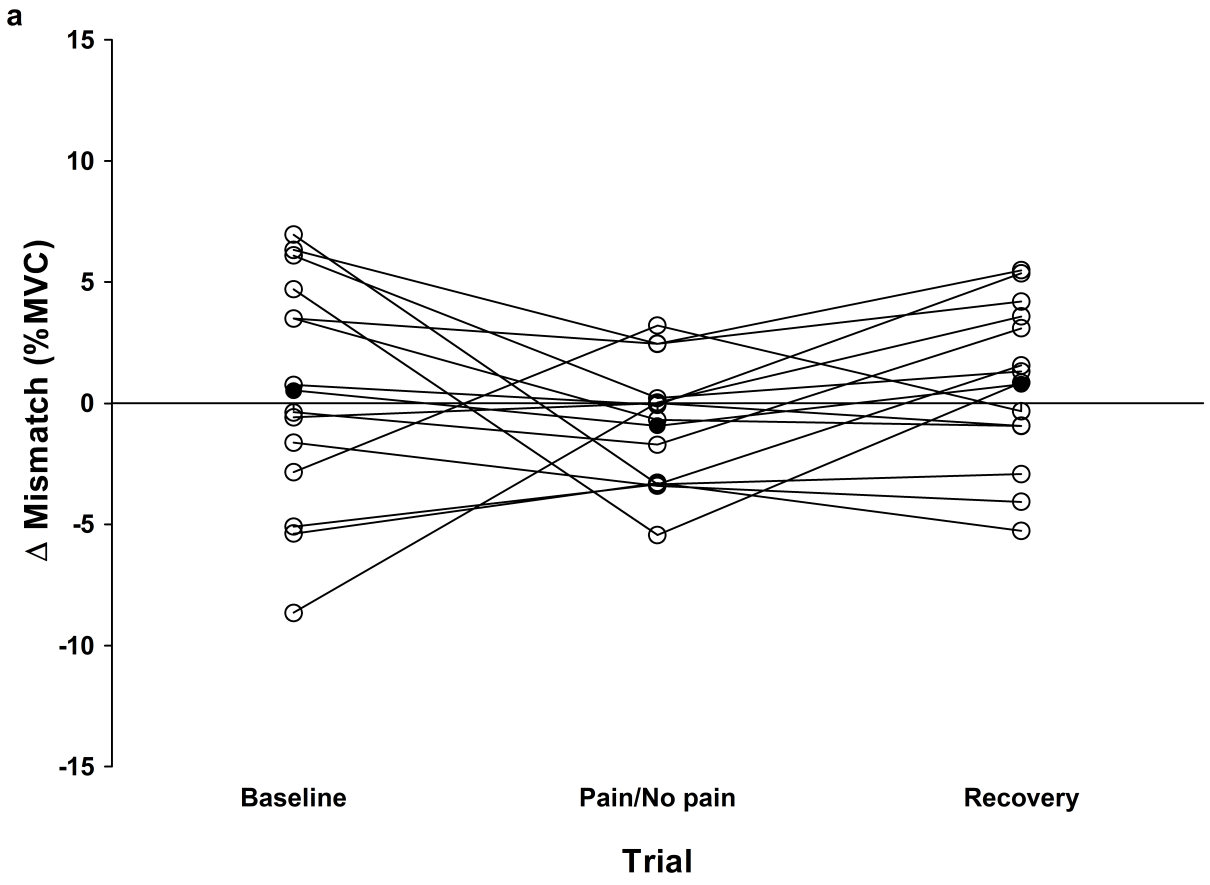
Pain/No pain
Recovery
Trial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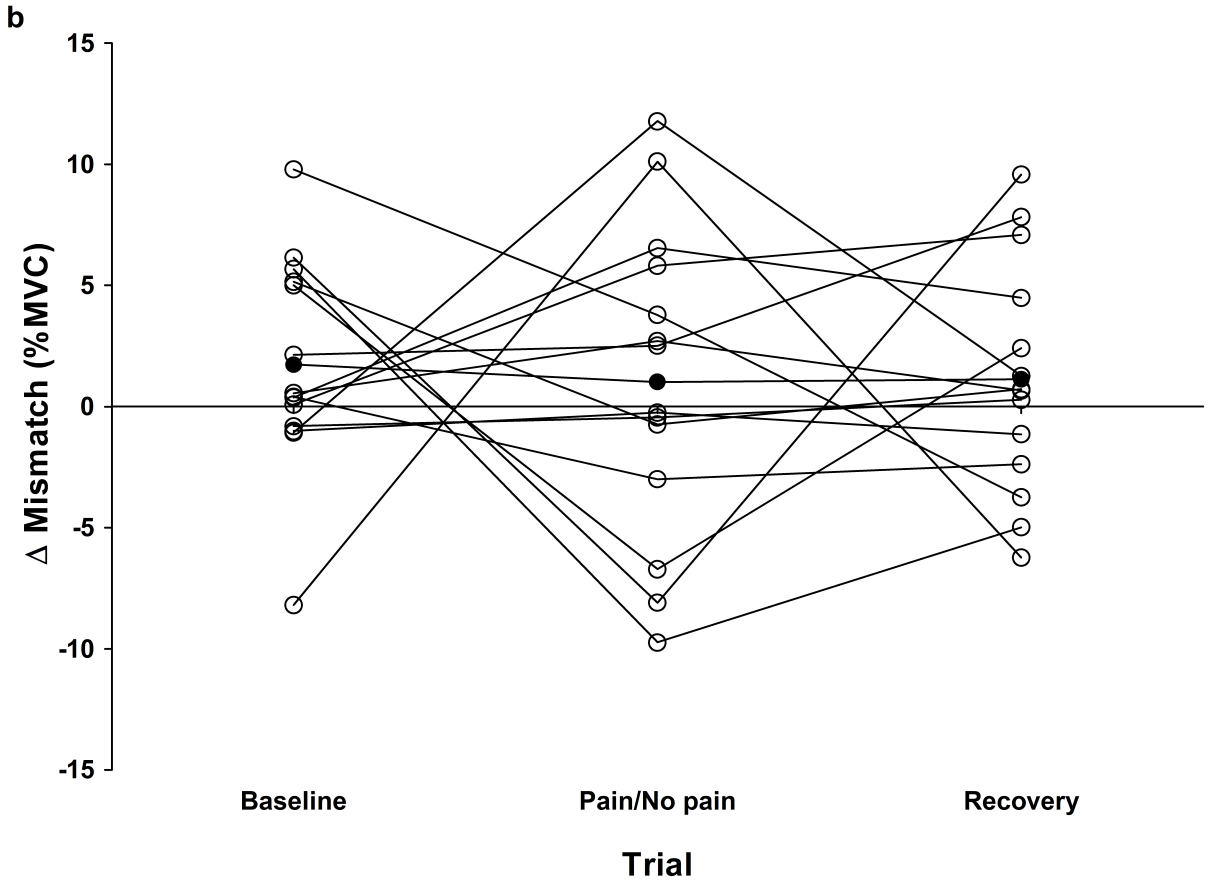
Δ Mismatch (%MVC)
Baseline
Pain/No pain
Recovery
Trial


b
15
10
5
0
-5
-10
-15
Δ Mismatch (%MVC)
Baseline
Pain/No pain
Recovery
Trial