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A task-relevant experimental pain model to target motor adaptation

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ABSTRACT:

Contemporary pain adaptation theories predict that motor adaptation occurs to limit pain. Current experimental pain models, however, do not allow pain intensity modulation according to one's posture or movements. We developed a task-relevant experimental pain model using low-frequency sinusoidal electrical stimuli applied over the infrapatellar fat pad. In fourteen participants, we compared perceived pain habituation and stimulation-induced artefacts in vastus medialis electromyographic recordings elicited by sinusoidal (4, 10, 20 and 50Hz) and square electrical waveforms delivered at constant peak stimulation amplitude. Next, we simulated a clinical condition where perceived knee pain intensity is proportional to the load applied on the leg by controlling sinusoidal current amplitude (4Hz) according to the vertical force the participants applied with their right leg to the ground while standing upright. Pain ratings habituated over a 60s period for 50Hz sinusoidal and square waveforms but not for low-frequency sinusoidal stimuli ($p<0.001$). EMG filters removed most stimulation artefacts for low-frequency sinusoidal stimuli (4Hz). While balancing upright, participants' pain ratings were correlated with the force applied by the right leg ($R^2=0.65$), demonstrating task-relevant changes in perceived pain intensity. Low-frequency sinusoidal stimuli can induce knee pain of constant intensity for 60s with minimal EMG artefacts while enabling task-relevant pain modulation when controlling current amplitude. By enabling task-dependent modulation of perceived pain intensity, our novel experimental model replicates key temporal aspects of clinical musculoskeletal pain while allowing quantification of neuromuscular activation during painful electrical stimulation. This approach will enable researchers to test the predicted relationship between movement strategies and pain.

KEY POINT SUMMARY:

- Motor adaptation is thought to be a strategy to avoid pain.
- Current experimental pain models do not allow to consistently modulate pain perception depending on one's movements.
- We showed that low-frequency sinusoidal stimuli delivered at painful intensity result in minimal habituation of pain perception (over 60s) and minimal stimulation artefacts on electromyographic signals.
- When the amplitude of the low-frequency sinusoidal stimuli was modulated based on the vertical force participants applied to the ground with the right leg while standing upright, we demonstrated a strong association between perceived pain and motor adaptation.
- By enabling task-relevant modulation of perceived pain intensity and the recording electromyographic signals during electrical painful stimulation, our novel pain model will permit direct experimental testing of the relationship between pain and motor adaptation.

RUNNING TITLE:

Motor adaptation to task-relevant pain: a novel experimental model.

Keywords: Pain, adaptation, electrical stimulation, EMG, knee

INTRODUCTION

It is widely acknowledged that pain changes the way we move. Contemporary pain theories predict that motor adaptation occurs to limit pain (Hodges & Tucker, 2011). Experimental pain approaches are appropriate to test predictions from these theories and provide insights into motor adaptation variability that are difficult to infer from clinical populations. The most commonly used experimental pain model consists in the injection of hypertonic saline solution in muscles (Zedka *et al.*, 1999; Birznieks *et al.*, 2008; Gallina *et al.*, 2018*b*; Martinez-Valdes *et al.*, 2020) or other soft tissues (Bennell & Hinman, 2005; Birznieks *et al.*, 2008; Tsao *et al.*, 2010; Gallina *et al.*, 2018*b*), which results in tonic pain that is not consistently modulated by how an individual moves. The pain experienced by individuals with musculoskeletal disorders, however, is alleviated or exacerbated by specific movements or postures (Lewis, 2015; Crossley *et al.*, 2016; Karayannis *et al.*, 2016; Madry *et al.*, 2016); this has prompted researchers to investigate sensorimotor adaptations during movements that specifically evoke pain (Wang *et al.*, 2018, 2021). Moreover, as the pain perception induced by hypertonic saline solution is not consistently modulated when motor adaptation occurs, motor adaptation may be altered if pain is exacerbated by specific movements and/or alleviated when an appropriate alternative motor strategy is identified. While hypertonic saline solution injections are appropriate to reproduce the physiological effects of tonic muscle pain, the motor adaptations observed following these injections may provide only partial information on how the sensorimotor system adapts to pain. Hence, there is a critical need for experimental pain models that evoke a pain perception modulated with movement/posture to better understand the relationship between pain and motor adaptation.

Electrical stimuli can activate nociceptors in non-muscular tissues (Koga *et al.*, 2005; Maffiuletti *et al.*, 2008), with stimulus intensity modulating pain perception (Laursen *et al.*, 1997). Brief trains of electrical stimuli (square waves; 100-200ms burst of 200-1000μs impulses delivered at ~50-200Hz) enable the characterization of altered neuromuscular activation in anticipation or in response to motion-induced pain (Moseley *et al.*, 2004; Moseley & Hodges, 2005; Tucker *et al.*, 2012; Schouppe *et al.*, 2020). However, repeated application of electrical painful stimuli may lead to habituation in pain perception (Ernst *et al.*, 1986; Eitner *et al.*, 2018), hence painful electrical

stimulation has mostly been used to reproduce phasic pain (Zedka *et al.*, 1999; Moseley *et al.*, 2004; Moseley & Hodges, 2005; Tucker *et al.*, 2012; Schouppe *et al.*, 2020). In addition, electrical stimuli create artefacts in electromyographic (EMG) recordings, preventing the quantification of muscle activation during the application of the painful stimuli (Zedka *et al.*, 1999; Moseley *et al.*, 2004; Moseley & Hodges, 2005; Tucker *et al.*, 2012; Schouppe *et al.*, 2020). Some of these limitations may be overcome by using low-frequency sinusoidal electrical stimuli because: 1- they preferentially activate skin polymodal C-fibres and silent nociceptors (Koga *et al.*, 2005; Matsumoto *et al.*, 2006; Jonas *et al.*, 2018) with minimal concurrent activation of larger afferents that may contribute to gating of nociceptive inputs (Melzack & Wall, 1965; Luz *et al.*, 2014; Löken *et al.*, 2017; Fernandes *et al.*, 2020) and 2- they can be delivered at frequencies below the bandwidth of the EMG (i.e. < 20Hz (De Luca *et al.*, 2010)), which may enable quantification of muscle activation during the painful stimuli after conventional filtering.

Here, we investigated the use of low-frequency sinusoidal electrical stimuli as an experimental model to induce task-relevant pain, where pain intensity is modulated by how an individual moves, thus reproducing temporal features of pain similar to those experienced by individuals with musculoskeletal disorders. Furthermore, we investigated if EMG signals with minimal stimulation artefact can be collected during painful stimulation elicited by low-frequency sinusoidal electrical stimuli. We hypothesized that, compared to high-frequency sine waves and square wave stimuli, low-frequency sinusoidal electrical stimuli would result in less habituation of pain intensity ratings over time. Second, we hypothesized that EMG signals would exhibit smaller stimulation artefacts (after filtering) for low-frequency sinusoidal electrical stimuli compared to square wave stimuli. Finally, we modulated the amplitude of low-frequency electrical stimuli based on the loading participants applied to the ground while balancing upright. We hypothesized that perception of pain would be modulated based on loading applied by participants to the ground.

METHODS

Participants

Fourteen healthy adult participants (8 males and 6 females) without lower limb pain and/or injury were recruited from the university community. Participants were excluded from the study is they had any history of knee surgery or musculoskeletal symptoms for which they sought medical care. Participants mean (± SD) age, height and weight were respectively 27.0 ± 4.1 years, 172±10 cm and 69.3±13.5 kg. The study conformed to the Declaration of Helsinki, except for registration in a database, and was approved by the University of British Columbia's Clinical Research Ethics Board (H17-02672). All participants gave written informed consent, acknowledging their right to withdraw from the experiment without consequences.

Study design

To explore the use of low frequency electrical stimuli as a pain model and address our hypotheses, three distinct experiments were conducted in the same session. In Experiment 1, we assessed the habituation of verbal pain ratings over a 60s period. In Experiment 2, we examined the possibility to remove artefacts induced by low frequency electrical stimuli in surface EMG signals recorded from the vastus medialis. In Experiment 3, we tested if low frequency sinusoidal electrical stimuli could induce task-relevant pain where the intensity of electrical stimuli applied to the knee pain depended on the load that participants applied to the ground with their right leg during quiet standing. Two participants were excluded from Experiments 2 and 3 due to technical issues with the data acquisition software. Data from another participant were excluded from Experiment 2 due to the electrical stimuli induced artefacts saturating of the EMG amplifier.

Painful electrical stimuli

Electrical stimuli were delivered through two surface electrodes (2.2 × 3.5 cm, H59P, Kendall, Covidien) placed over the skin of the medial (cathode) and lateral (anode) aspects of the infrapatellar fat pad of the right knee. The right leg was the preferred leg to kick a ball for 13 out 14 participants. We identified the infrapatellar fat pad through manual palpation and chose this location because the fat pad is densely innervated by nociceptors (Bohnsack *et al.*, 2005) and is considered to be one of the potential sources of pain in knee musculoskeletal disorders (Ioan-Facsinay & Kloppenburg, 2013; Cowan *et al.*, 2015; de Vries *et al.*, 2020). Compared to previous studies where both stimulating electrodes were placed on the medial side of the knee

(Tucker *et al.*, 2012), we positioned the electrodes on the medial and lateral aspects of the fat pad to direct the current through a larger portion of the tissue, possibly stimulating a larger number of nociceptors. Electrical stimuli were administered using a constant-current stimulator (Digitimer DS5 Isolated Bipolar Constant Current Stimulator, Welwyn Garden City, Hertfordshire, UK) controlled using an analog signal generated by a NI multifunction data acquisition board (PXI-6289 National Instruments, Austin, TX, USA). This analog signal was created using a digital-to-analog port of the data acquisition board and digitized with 16 bits at 2048Hz. Using a custom virtual instrument (LabVIEW 2013; National Instruments, Austin, TX, USA), we programmed and delivered painful electrical stimuli with 1ms square waves at 40Hz or sinusoidal waveforms at four different frequencies (4Hz, 10Hz, 20Hz, and 50Hz). These stimulus parameters were chosen to test the effects of waveform type and frequency on 1) the habituation of pain intensity ratings over time (Experiment 1) and 2) the induced stimulation artefacts on the EMG signals (Experiment 2). For Experiments 1 & 2, the order of the painful electrical stimuli was randomized between participants. For Experiment 3, experimental pain was delivered using only the 4Hz electrical stimuli because they have been proposed to preferentially activate C-fibers (Jonas *et al.*, 2018), and because minimal habituation was observed in preliminary data.

Data collection

Myoelectric activity was recorded from the right vastus medialis (Figure 1), a muscle commonly assessed in clinical and experimental pain studies (Chester *et al.*, 2008; Gallina *et al.*, 2018*b*). The skin over the vastus medialis was cleaned with abrasive gel (Neuprep, Weaver and Co, Aurora, CO) and shaved when necessary. Muscle activity was recorded with a high-density surface EMG (HDsEMG) of 64 electrodes arranged in 5 columns and 13 rows spaced by 8 mm (semi-disposable adhesive matrix; model ELSCH064, OTBioelettronica, Torino, Italy). The electrode grid was placed orienting the columns along the approximate muscle fiber orientation. The same investigator was responsible for the placement of the grid for all participants to minimize variability. Electrodes in the most distal part of the HDsEMG grid were located ~3 cm from the cathode electrode. Reference electrodes for the HDsEMG were placed over the right iliac crest. HDsEMG signals were amplified (×100 or ×200, to minimize the possibility of saturation in EMG signals), filtered (10-500Hz), digitized in monopolar mode at 2048Hz using a 12-bit A/D converter (128-channel EMG-USB; OTBioelettronica, Torino, Italy). The analog

signal driving the isolated electrical stimulator was recorded simultaneously with the NI multifunction data acquisition board and the HDsEMG amplifier to synchronize the data.

For Experiments 2 & 3, we used force plates (AMTI model OR6-7-1000, Watertown, MA, USA) to compute maximal voluntary force exerted by the knee extensors and vertical forces applied by the participants while balancing. For Experiment 2, participants were seated comfortably with their knee angle at ~90 degrees. We secured their right ankle using a strap anchored to the force plate with a bolt. We estimated knee extension force using the magnitude of the horizontal forces applied to the force plate. In Experiment 3, participants stood upright and maintained standing balance. We calculated the force applied by their right leg to the ground using the vertical forces acting on the force plates under their feet. Forces plate signals were amplified (×4000, AMTI model MSA-6, Watertown, MA, USA) and the voltage signals were digitized at 2048Hz using hardware single point data acquisition programmed with a custom LabVIEW virtual instrument (LabVIEW, National Instruments; and PXI-6289, National Instruments; 2048Hz). For each acquired data point, the custom LabVIEW virtual instrument transformed the voltage signals acquired from the force plate into calibrated forces and moments.

Experiment 1: Habituation of pain ratings to electrical stimuli

The aim of Experiment 1 was to assess the effects of stimulus waveform and frequency on habituation of pain ratings to the electrical stimuli. Participants sat comfortably on a wooden box without back support and with their knee flexed at ~90 degrees. In this posture, participants received five different types of electrical stimuli (1ms square waves at 40Hz and sinusoidal stimuli at 4Hz, 10Hz, 20Hz and 50Hz). The intensity of each electrical stimulus was adjusted to induce a moderate pain intensity (3/10) measured using a verbal numerical rating scale (NRS), anchored between 0 (no pain) and 10 (worst pain possibly imaginable). We targeted a pain level of 3/10 because it is the cut-off value commonly used to determine if a participant has patellofemoral pain in clinical studies (Esculier *et al.*, 2018; Gallina *et al.*, 2018*a*; Maclachlan *et al.*, 2018). In addition, a pain level of 3/10 is similar to what participants reported in other studies where knee pain was induced by injecting hypertonic saline solution in the infrapatellar fat pad (Poortvliet *et al.*, 2015; Salomoni *et al.*, 2016; Gallina *et al.*, 2018*b*). The stimulation intensity was determined using an ascending protocol involving 2s of electrical stimulation and ~5s of

rest. The ascending protocol started with a 0.1 mA baseline stimulus amplitude for all participants and increased with steps of 0.5 mA until participants perceived a pain of 1/10. Then, the amplitude of the stimulation increased in steps of 0.1 mA until participants reported an intensity of 3/10. Verbal pain ratings were collected during continuous painful electrical stimulation for 60s. Participants reported their perceived pain intensity using the NRS at 5s, 10s after the start of the stimulation and every 10s until the end of the stimulation. At the end of the 60s stimulation period, participants were asked to draw the localisation of the pain they experienced on two knee schematic drawings depicting an anterior and a transversal view of the knee (Figure 2). Participants also indicated all of the words that best described their perceived pain using the McGill Pain Questionnaire to evaluate the quality of their pain (Melzak, 1975). This protocol was repeated for each electrical stimulation type; stimuli were presented in a randomized order between participants.

Experiment 2: EMG recordings during the electrical stimuli

The aim of experiment 2 was to investigate if low frequency sinusoidal electrical stimuli delivered at painful intensities enabled the measurement and quantification of EMG signals with minimal artefacts. In a seated posture with their knees at ~90 degrees, participants performed three isometric maximal voluntary contractions (MVC) of their right knee extensors for approximately 5s while the experimenter provided verbal encouragement. A 1-min rest period was provided between each MVC to limit muscle fatigue. The highest knee extension force value was considered the MVC for the rest of the protocol. Participants were asked to generate a 10% MVC target force. They were provided visual feedback of their force using a 22 inches computer screen positioned approximately 1m in front of them. This 10% MVC target force was chosen to elicit consistent but low-level EMG signals to determine if muscle activation could be quantified for levels expected for tasks of daily living. For each stimulation waveform and frequency, participants performed a 20s trial consisting sequentially of: i) a 5s rest period without stimulation; ii) a 5s rest period with moderate pain (3/10 NRS) induced by the electrical stimulation; iii) a 5s of active period where participants generate a 10% MVC without stimulation; and iv) a 5s active period where participants generated a 10% MVC with moderate pain induced by the electrical stimulation. The rest and active periods with and without the electrical stimuli were performed to determine the effects of the electrical stimuli during

background and low-level muscle activation. This protocol was repeated for each waveform profile (sinusoidal 4Hz and 10Hz; square waves) and the order of the stimuli was randomized between participants. Sinusoidal stimulation at higher stimulation frequencies (20Hz and 50Hz) was not considered in this analysis because these frequencies are within the EMG signal bandwidth, therefore conventional EMG filtering is not expected to remove the stimulation artefact.

Experiment 3: Task-relevant modulation of perceived pain intensity

The aim of Experiment 3 was to investigate whether 4Hz low-frequency sinusoidal stimuli can induce a perception of pain that is modulated in a task-relevant manner. As a secondary aim, we examined whether participants adapted their behaviour in response to the painful stimuli modulated in a task-relevant manner. In Experiment 3, we used low-frequency painful electrical stimulation to induce knee pain during a daily living activity (quiet stance) to understand how participants adapt to knee pain in an ecological task where they are not instructed to precisely regulate muscle force production (in contrast to force feedback used in Experiment 2). Participants stood upright relaxed with their feet on two separate force plates. Stance width was standardized to each participant's foot length. The baseline force applied by their right leg to the ground was calculated as the vertical force applied by their right foot on the force plate over 60s of quiet standing divided by the total vertical force applied by the two feet on the force plates. This measure of relative load applied by the right leg to the ground was used to determine a threshold to modulate the painful stimulation intensity in following trials. We chose these tasks and procedures to modulate the intensity of the electrical stimuli to induce a perception of pain intensity that was graded to the load applied by the painful leg on the ground. As participants maintained upright balance, we controlled the amplitude of the painful stimulation to modulate perceived pain intensity. For each data sample (digitized at 2048Hz), the amplitude of the electrical stimuli was modulated in real-time by multiplying a 4Hz sinusoidal waveform of unitary amplitude by a signal proportional to the instantaneous magnitude of the load on the right leg (Figure 1). We established two boundaries for the amplitude of the 4Hz electrical stimuli based the perception of pain threshold and the 3/10 painful intensity (NRS) quantified for each participant in Experiment 1. When the vertical force applied by the right leg to the floor was equal to or higher than baseline, the amplitude of the painful electrical stimuli corresponded to

the one chosen by the participant to induce a pain perception of 3/10 NRS (7.4±2.2 mA). When participants shifted their weight to the left leg and reduced the load on the right leg relative to load applied by both legs by 2.5% or more (equivalent to a 5% decrease in vertical force applied by the right leg on the ground), the amplitude of the electrical stimuli was set at an intensity 10% lower than their identified pain threshold (4.5±1.9 mA), hence generating a non-painful sensory perception. When the relative loading on the right leg was between 100% and 97.5% of the baseline value, the amplitude of the painful electrical stimuli varied linearly between intensities required to generate no pain sensation and 3/10 (NRS) according to the instantaneous load applied by the participants' right leg during standing balance.

We asked participants to balance upright under two conditions performed sequentially to explore if they could discover that shifting their body weight toward the left leg modulated their pain perception. During the first condition, one of the experimenters told participants: "stimulation amplitude and pain intensity may change during the trial", but participants were unaware of the possibility to modulate the stimulation amplitude. These instructions were provided to the participants in order to avoid them thinking that the experimental procedures were not working because they knew they were participating in a pain experiment and expected to experience pain. During the second condition, participants received the following instructions: "there is a way for you to decrease the pain". Hence for this trial, participants were aware that there was a strategy they could use to reduce their pain intensity, but they were not told what the strategy was. During both conditions, participants rated their pain intensity using the NRS after 5s, 10s, and at every 10s interval thereafter to determine if they modulated the intensity of their pain perception during the 60s standing balance trials. At the end of the second trial, participants were also asked to report which strategy they used to reduce their perceived pain intensity.

Data analysis

Drawings of the painful areas were digitized for each participant. We averaged spatial distribution of painful areas across participants to create heatmaps to visually describe the localization of pain across different electrical stimuli. From the McGill pain questionnaire, we reported the percentage of participants choosing specific adjectives to describe their subjective pain experience.

As the amplitude of the stimulation-induced artefact on the EMG signals depends on the distance from the stimulation to the recording locations (Petrofsky *et al.*, 2009), we simulated two bipolar EMG detection sites over the muscle belly. We computed the difference between the average monopolar signals from groups of 6 channels in a proximal and a distal location within the electrode grid (location described in Figure 1). We digitally band-pass (20–400Hz) filtered (8th-order dual-pass zero-phase Butterworth) all EMG signals because analog filters typically exhibit slow roll-off and we wanted to minimize the presence of the stimulus-induced artefacts in the EMG signals using commonly filters (De Luca *et al.*, 2010). We also used a 30-400Hz band-pass filter (8th-order dual-pass zero-phase Butterworth) to assess the effects of a high-pass filter with a higher cut-off. Notch filters (8th-order dual-pass zero-phase Butterworth; 59-61Hz and multiples up to 472-488Hz) were used in all signals to eliminate the 60Hz power line interference and its harmonics. We quantified the amplitude of vastus medialis EMG by computing the root mean square (RMS) value over a 4s window centered in the middle of the 5s stimulation period or with a 4s window ending 500ms before the start of the stimulation. Amplitude spectra were also computed using Fast Fourier Transform to visually assess the presence of stimulation artefacts.

To quantify changes in load applied by the right leg to the ground through the duration of the 60s standing balance trial, we averaged the magnitude of the vertical force applied by the right leg to the ground for each 5s period preceding verbal pain rating (5s, 10s, and every 10s thereafter). To make the vertical force data relevant to the values used to modulate the amplitude of the electrical stimuli, we normalized the magnitude of the vertical force by the average vertical force measured during the 60s of quiet standing. We quantified the load applied by the right leg to the ground with respect to baseline values because the variations in vertical forces applied by both feet to the ground during trials was minimal and to simplify the presentation of the results. Hence, vertical forces are presented as percentage of baseline where values higher or lower than 100% indicate increased or decreased force applied by the right leg to the ground compared to baseline, respectively and a 5% modulation of the vertical force applied by the right leg led to a current amplitude targeting a non-painful sensation. All data analyses were performed in Matlab (2018b version, Mathworks, Natick, MA, USA).

Statistical analysis

Statistical analyses were performed in SPSS statistics for Windows, version 26 (SPSS Inc., IBM Corp., Armonk, N.Y., USA). Parametric and non-parametric tests were chosen based on the data distribution (Shapiro-Wilk test). As most participants reported pain intensity values rounded to the nearest 0.5, non-parametric statistics were used for the NRS data. A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated. We first investigated differences in stimulation intensity between electrical stimuli waveforms necessary to induce a moderate pain of 3/10 (measured during the ascending protocol) using a one-way repeated measures ANOVA. Decomposition of the main effect was performed using pairwise comparisons with Bonferroni-corrected paired T-tests (all pairwise comparisons).

To address our first hypothesis, we assessed the effects of Stimulation waveform and Time on pain ratings reported at 5s and every subsequent 10s. As we expected a Stimulation waveform × Time interaction, and we are not aware of non-parametric two-way repeated measures ANOVA procedures that test interaction effects, we performed an ordinal logistic regression (Generalized Estimating Equations for repeated measures in SPSS). When present, we decomposed the interaction by identifying which Stimulus waveforms resulted in decrease in pain ratings over time using separate Friedman tests for each Stimulus waveform. For Stimulus waveforms exhibiting a main effect, we then used Dunn post-hoc tests with Bonferroni correction (6 comparisons) to determine which Time points were significantly different from the first pain rating (5s).

To address our second hypothesis, we compared the RMS values of EMG signals quantified at rest and during a 10% MVC with and without the electrical stimulus. We performed separate paired Wilcoxon tests comparing EMG amplitude before and during painful electrical stimulation for each electrode location (proximal or distal), muscle contraction (rest or active) and high-pass filter cut-off values (20Hz or 30Hz).

To address our third hypothesis, we first examined if the reported pain ratings during the standing balance task were modulated with Awareness of the possibility to modulate pain (aware and unaware) and Time (5s, 10s, and every subsequent 10s). We further aimed to characterize if a task-relevant painful electrical stimulus modulated the standing balance behaviour across Awareness conditions (aware and unaware) and Time (5s, 10s, and every subsequent 10s) by

comparing the magnitude of vertical force applied by the right leg to the ground during painful stimulation. Because the magnitude of vertical force was not normally distributed, we applied a rank transformation. For both magnitude of vertical force and reported pain, we used ordinal logistic regression to assess the interaction between the Time and Awareness factors (Generalized Estimating Equations for repeated measures in SPSS). When an interaction was present, we decomposed it using separate Friedman tests to assess which Awareness conditions resulted in adaptation over time; then, we used Dunn post-hoc tests with Bonferroni correction (6 comparisons) to determine which Time points were significantly different from the first pain rating (5s). Next, we quantified the association between the vertical force applied by the right leg to the ground and reported pain ratings at 60s (when participants were expected to show the largest pain modulation) using a Spearman correlation test. A significance level of 0.05 was used for all analyses and data are reported as mean and standard deviation when normally distributed, or otherwise as median and interquartile range.

RESULTS

Experiment 1: Habituation of pain ratings to electrical stimuli (N=14 participants)

The amplitude of the electrical stimuli required for participants to report a pain intensity of 3/10 was: square waves, 10.5±4.3 mA; 4Hz, 7.4±2.2 mA; 10Hz, 6.5±2.0 mA; 20Hz, 6.2±1.4 mA; 50Hz, 6.0±2.3 mA. The stimulation intensity was 29.2-42.8% lower for sinusoidal waveforms compared to 1ms square waves delivered at 40Hz ($F_{(1.9, 24.4)} = 16.7$, $p<0.0001$; Bonferroni-corrected p values for square waves compared to: 4Hz, p=0.046; 10Hz, p=0.003; 20Hz, p=0.005; 50Hz, p=0.002). Furthermore, the amplitude of 50Hz sinusoidal electrical stimuli was 19.1±23.9% lower than 4Hz sinusoidal electrical stimuli to elicit a similar perception of pain (Bonferroni-corrected p=0.049).

To assess the effects of electrical stimulus waveform and frequency on habituation of pain ratings, participants rated their perceived pain intensity while exposed to various stimuli for 60s. Habituation of perceived pain intensity over 60s was observed only for higher frequency electrical stimuli sinusoidal or 1ms square waveforms (Figure 3; Stimulation waveform × Time

interaction: $\chi^2_{(13)}$=153.97, p<0.0001). To characterize these effects, decomposition of the interaction for each electrical stimulus waveform revealed habituation in perceived pain intensity over time for the 50Hz sinusoidal electrical stimuli ($\chi^2_{(6)}$=29.43, p<0.0001) and the 1ms square waves at 40Hz ($\chi^2_{(6)}$=36.723, p<0.0001). For the 50Hz sinusoidal stimuli, participant reported 20.5±19.6% lower pain intensity at 40s (z=2.68, Bonferroni-corrected p=0.006) that remained stable up to the 60s time point (21.7±24.6% at 60s; z=2.50, Bonferroni-corrected p=0.008). For the 1ms square waveforms, participants reported 24.4±26.1% lower pain ratings at 40s after the start of the stimulation (z=2.61, Bonferroni-corrected p=0.006); the participants' average reported pain intensity continued to decrease for the rest of the 60s trial until it reached 64.6±32.9% of the initial value at 60s (z=4.33, Bonferroni-corrected p<0.0001). In contrast, participants reported consistent perceived pain intensity, showing on average no clear signs of habituation or sensitization, during the 60s application of the 4Hz ($\chi^2_{(6)}$=4.79, p=0.570), 10Hz ($\chi^2_{(6)}$=10.59, p=0.102) and 20Hz ($\chi^2_{(6)}$=9.33, p=0.156) sinusoidal electrical stimuli. These results show that low frequency sinusoidal electrical stimuli can induce a stable perception of pain for at least a 60s duration.

When describing their pain sensation, participants used most frequently the descriptors "flickering, quivering, pulsing, throbbing, beating, pounding" (7/14 square waves; sinusoidal stimuli: 8/14 4Hz, 11/14 10Hz, 7/14 20Hz, 6/14 50Hz), followed by "tingling, itchy, smarting, stinging" (7/14 square waves; sinusoidal stimuli: 5/14 4Hz, 7/14 10Hz, 5/14 20Hz, 8/14 50Hz) categories. Participants reported the temporal aspect of their pain as "rhythmic" for low-frequency sinusoidal stimulation (8/14 at 4Hz; 7/14 at 10Hz) and "continuous" for 20Hz and 50Hz sinusoidal electrical stimuli (8/14 for 20Hz; 7/14 for 50Hz). Pain associated with the 1ms square wave stimuli was rated as continuous (4/14), transient (3/14) or rhythmic (3/14). The average pain drawings (Figure 2) revealed that most participants reported pain localized around or just lateral to the electrode placed on skin over the medial fat pad for the 1ms square waves and 4Hz sinusoidal electrical stimuli or between the two stimulation electrodes for the 10Hz, 20Hz and 50Hz sinusoidal stimuli. Drawings to localize pain in the transversal knee section showed that most participants reported superficially localized pain, close to the electrode location. Up to half of the participants also reported pain areas extending into the medial side of the patellofemoral joint, particularly for the 10Hz and 20Hz sinusoidal electrical stimuli.

Experiment 2: EMG recordings during the electrical stimuli (N=11 participants)

Having established that low frequency sinusoidal electrical stimuli can induce a stable perception of pain, we quantified the presence of stimulation artefacts on EMG signals recorded from the vastus medialis while participants were at rest. Assessing the presence of stimulation artefact in the absence of muscle activation was important to determine changes in amplitude of the EMG signal due to stimulation artefact alone, regardless of potential changes in muscle activation induced by pain. We also investigated the presence of stimulation artefacts when participants performed a 10% MVC knee extension to determine if muscle activation levels could be estimated during periods of low frequency electrical stimuli delivered at painful intensities. Painful electrical stimulation resulted in large stimulation in the EMG signals before filtering both for the proximal (peak-peak values, median [25^{th}-75^{th} percentiles]; square waves: 2.1 [1.89-2.95] mV, 4Hz: 1.94 [1.84-3.17] mV, 10Hz: 2.28 [2.09-2.51] mV) and distal (square waves: 3.02 [0.78-5.81] mV, 4Hz: 2.82 [0.46-7.75] mV, 10Hz: 4.93 [1.45-5.59] mV) locations. After filtering, the presence of stimulation artefacts in the EMG signals depended on the electrical stimulus waveform used to induce a painful perception. Visual analysis of the EMG amplitude spectra revealed that sinusoidal electrical stimuli induced a peak in power centered around the stimulation frequency whereas the 1ms square wave electrical stimuli caused several peaks in amplitude across the frequency spectra. Conventional digital high-pass filtering (20Hz or 30Hz) of the EMG signals largely attenuated the induced artefacts in EMG amplitude for the sinusoidal stimuli but not for the 1ms square wave stimuli (Figure 4). Consequently, regardless of the digital high-pass filter used (20Hz or 30Hz), artefacts introduced by the 1ms square wave electrical stimuli resulted in notable increases in EMG RMS amplitude for the rest and 10% MVC condition (median increase: ~30 µV for the distal location, ~300 µV for the proximal location; all data and statistical comparisons are presented in Table 1). In contrast, EMG artefacts induced by sinusoidal electrical stimuli were largely removed with digital high pass filters, resulting in modest increases (< 1.1 µV or 2.8 µV for the 4Hz and 10Hz stimuli, respectively; Table 1) in the median RMS EMG amplitude across conditions. In general, the RMS EMG amplitudes estimated during the 4Hz sinusoidal electrical stimuli were similar to those measured without electrical stimulation for the rest and 10% MVC conditions. Applying a 30Hz high-pass

filter to the EMG signals recorded during the 4Hz sinusoidal stimuli resulted in the largest reduction in the stimulus-induced artefacts (increase in RMS of ~0.5 μV) for both calculated recording electrode locations but the differences between EMG RMS amplitude estimated with both high-pass filters (20Hz or 30Hz) were small (~1 μV; Table 1). These results confirm that muscle activation patterns can be evaluated during painful stimuli with minimal interference from artefacts induced by 4Hz and 10Hz sinusoidal electrical stimuli in the EMG signals when using conventional EMG digital filters.

Experiment 3: Task-relevant modulation of perceived pain intensity (N=12 participants)

To determine if electrical stimuli can be used as an experimental pain model to replicate the movement or posture dependent pain modulation observed in certain clinical disorders, we quantified perception of pain of healthy participants standing upright while they were exposed to 4Hz low-frequency sinusoidal electrical stimuli. The amplitude of the electrical stimuli was modulated based on the loading participants applied to the ground with their right leg. A significant Awareness × Time interaction ($\chi^2_{(6)}$=13.73, p=0.033; Figure 5) indicated that the pain ratings decreased differently over time when participants were aware or unaware that they could modulate their pain intensity. Although a significant adaptation over time was identified both in the aware ($\chi^2_{(6)}$=36.30, p<0.0001) and in the unaware ($\chi^2_{(6)}$=36.88, p<0.0001) conditions, significant decrease in reported pain was observed earlier in the aware condition (51.2±39.2% of the initial value at 40s; z=3.02, p=0.012) than in the unaware condition (62.3±37.2% of the initial value at 50s; z=3.92, p=0.012). The lower pain ratings observed at 5s during the aware condition can be explained by the rapid adaptation observed in two participants who identified the correct strategy during the preceding unaware condition and started the task by standing mostly on their left leg. When excluding these two participants, the average pain rating at 5s during the aware condition was 3.0±0.5 but the general outcomes from statistical analyses did not change. At the end of the aware task, we prompted participants to describe the strategy they used to modulate their perception of pain. Participants reported using different strategies, including increasing the load on their left leg (N=3), contracting their lower limb muscles (N=3),

relaxing their lower limb muscles (N=2), flexing their knee joint (N=2), bending forward (N=1) and positive thinking (N=1).

We then characterized if the task-relevant painful electrical stimulus modulated the standing balance behaviour in participants that were aware or unaware that they could modulate their pain intensity. On average, participants decreased the load they applied to the ground with their right leg over time (Figure 6). This relative shift in loading between their left and right legs, however, occurred with a different time course when participants were aware that pain intensity could be modulated (significant Awareness × Time interaction; $\chi^2_{(6)}$=22.54, p=0.001). On average, participants reduced the load applied by their right leg to the ground over time in the aware condition ($\chi^2_{(6)}$=26.86, p=0.0001), but not in the unaware condition ($\chi^2_{(6)}$=6.71, p=0.348). In the aware condition, participants reduced the load on their right leg by at least 13.9±19.9% at all time points starting from 40s in the aware condition (40s, p=0.0048; 50s, p=*0.084*; 60s, p=0.0014; Figure 6). To examine if the intensity of pain reported by the participants depended on their standing balance behaviour, we quantified the association between their reported pain ratings with the vertical force applied by their right leg to the ground (i.e. the signal controlling the amplitude of the low frequency sinusoidal electrical stimuli). When using the data from the aware condition at 60s (i.e. when participants exhibited the largest pain adaptation), a correlation analysis (Figure 5) confirmed that a large portion of the variance (R^2=0.65, p=0.001) in reported pain was associated with the amplitude of the vertical force applied by the right leg to the ground.

DISCUSSION

In the present study, we developed a novel experimental pain model where low-frequency sinusoidal electrical stimuli were used to induce task-relevant pain. We investigated whether sinusoidal electrical stimuli delivered at frequencies ranging from 4Hz to 50Hz and 1ms square wave stimuli induce a stable pain perception over a 60s period, allow the quantification of muscle activity during the application of the painful stimuli and induce task-relevant modulation in perceived pain intensity. We showed that

low-frequency sinusoidal electrical stimuli (4Hz, 10Hz, 20Hz) resulted in minimal or no habituation in perceived pain intensity over a 60s period whereas 50Hz sinusoidal electrical stimuli and 1ms square waves delivered at 40Hz induced rapid habituation in perceived pain rating. Low-frequency sinusoidal electrical stimuli (4Hz or 10Hz) applied at painful intensity enabled the quantification of EMG signals amplitude with minimal stimulation artefacts when a conventional digital high-pass filter was applied to the signals. When participants stood upright and controlled the electrical stimulus amplitude by modulating the vertical force they applied to the ground with their right foot, they reduced the load on their right leg only when they were aware they could decrease pain perception but rated pain lower over a 60s period in both the aware and unaware conditions (pain modulation occurred 10s earlier when aware). In addition, we observed an association between the standing balance task (loading on the right leg) and pain intensity reported by participants when they were aware they could modulate the painful stimulus. Altogether, our results support the use of low-frequency sinusoidal electrical stimulation as novel experimental model to induce task-relevant musculoskeletal pain.

We observed habituation in reported pain ratings over 60s when the painful electrical stimuli were delivered using 1ms square waves and 50Hz sinusoidal electrical stimulation, but only minimal habituation for frequencies ≤20Hz. Higher frequency sinusoidal electrical stimuli may activate a larger number of non-nociceptive afferents, which contribute to gating of the nociceptive afferent information (Melzack & Wall, 1965; Luz *et al.*, 2014; Löken *et al.*, 2017; Fernandes *et al.*, 2020). Also, nociceptors in rats (Raymond *et al.*, 1990; Gee *et al.*, 1996) and humans (Serra *et al.*, 1999) habituate to painful stimuli applied at frequencies higher than 10-20Hz. These higher frequency electrical stimuli result in the slowing of neural conduction velocity and eventually failure of impulse conduction if applied at frequencies above 10-20Hz (Herrero *et al.*, 2000). The absence of habituation during low-frequency sinusoidal electrical stimulation contrasts with previous reports of progressively lower pain ratings in healthy participants during continuous stimulation at 4Hz (Jonas *et al.*, 2018). A possible reason for the differences between studies is the location of the painful stimulation and type of receptors targeted. Jonas et al. (Jonas *et al.*, 2018) used small (0.04cm^2) and closely spaced electrodes to preferentially stimulate skin nociceptors (Klein *et al.*, 2004; Lelic *et al.*, 2012). We used larger electrodes (7.7cm^2, to minimize the contribution of skin receptors) positioned ~4cm apart to

direct the current through the infrapatellar fat pad, a tissue highly innervated by nociceptors (Bohnsack *et al.*, 2005) and of clinical relevance for knee musculoskeletal pathologies (Ioan-Facsinay & Kloppenburg, 2013; Cowan *et al.*, 2015; de Vries *et al.*, 2020). The observation that several participants reported pain deep within the joint and described their pain as 'throbbing, beating, pounding' suggests that our methods effectively targeted nociceptors in the infrapatellar fat pad, together with more superficial skin nociceptors. While our results demonstrate that it is possible to induce moderate knee pain intensity (NRS = 3/10) for up to 60s without habituation using low-frequency sinusoidal electrical stimuli, we recognize these observations may be specific to the location of stimulation (i.e. infrapatellar fat pad).

Compared to 1ms square waves, sinusoidal electrical stimuli applied at painful intensities resulted in negligible stimulation artefacts in the EMG recordings (generally <1μV RMS). As expected, the smallest stimulation artefacts were observed for the combination of lowest stimulation frequency (4Hz), higher high-pass filter (30Hz) and larger distance between recording and stimulating electrodes. The negligible stimulation artefact we observed indicates that low-frequency sinusoidal electrical stimuli allows the assessment of neuromuscular strategies using surface EMG during painful stimulation, even in muscles in close proximity to the electrical stimulus location. Hence, our approach provides an important advantage over brief trains (i.e.: 100 ms) of square wave electrical stimuli that induce EMG artefacts limiting the analysis of neuromuscular activation to periods before or after the stimuli (Moseley *et al.*, 2004; Moseley & Hodges, 2005; Tucker *et al.*, 2012; Schouppe *et al.*, 2020). Despite this key methodological advance for EMG recoding during painful electrical stimuli, careful considerations regarding the characteristics of the EMG amplifier, especially gain and input range of the A/D board, are needed to avoid its saturation during data acquisition (we excluded data from a participant for this reason). Future studies should investigate whether artefacts on the EMG signals can be removed when low-frequency sinusoidal stimulation is used to induce pain in body regions other than the skin on the fat pad.

We investigated the possibility to induce task-relevant pain by modulating the intensity of the painful electrical stimulation according to the load participants applied on

the ground with their right leg during quiet standing. This condition was designed to simulate clinical disorders where knee pain is exacerbated by joint loading. When participants were not aware that the intensity of the painful stimulation could be modulated, the load applied to their painful (right) leg did not change over time. Supporting our hypothesis, when participants were aware they could decrease their pain sensation, they decreased the vertical force applied by their right leg to the ground from 40s onwards (7/12 participants) and this was associated to a decrease in reported pain ratings over time. Five participants were unable to adopt a strategy to unload the right leg, and hence decrease the intensity of the painful stimulation. These findings are in line with the strategy reported by participants to decrease their pain intensity: most of them (9/12) did not consciously identify the correct strategy to minimise their pain but they decreased loading on the right leg. In line with these differences in motor adaptation, reported pain decreased earlier in the aware than in the unaware condition. The strong correlation (R^2=0.65) between motor adaptation (weight on the right leg) and reported pain intensity in aware participants demonstrates that low-frequency painful electrical stimuli can induce task-relevant pain, where pain intensity is modulated by their standing balance behaviour. This is an important advance for experimental pain models attempting to mimic pain modulation commonly observed in musculoskeletal disorders (Lewis, 2015; Crossley *et al.*, 2016; Karayannis *et al.*, 2016; Madry *et al.*, 2016) that cannot be obtained with standard methods such as injections of hypertonic saline solution. While delayed onset muscle soreness and nerve growth factor injections elicit a painful sensation that is modulated by muscle contraction/stretching (Svensson *et al.*, 2003; Prasartwuth *et al.*, 2005; Hedayatpour *et al.*, 2008; Schabrun *et al.*, 2016; Abboud *et al.*, 2019), these experimental pain models are currently limited to muscle pain. In addition, they do not allow the investigator to control what drives the painful stimulation (e.g.: weight applied by the right leg in our study) and it is challenging (if not impossible) to carefully regulate the intensity and duration of the painful stimulation.

Despite the many advantages and potential applications of low-frequency sinusoidal electrical stimulation as a pain model, there are some limitations to this model. First, our proposed experimental pain model using electrical stimuli lacks tissue spatial selectivity and it is inadequate as a muscle experimental pain model due to the potential occurrence of muscle twitches. Both of these issues can be addressed using hypertonic saline solution as a pain model,

highlighting that careful experimental considerations are required when choosing a pain model. Second, despite removing most of the stimulation-related artefacts during the painful stimuli, we observed artefacts in the EMG signals at the start and at the end of the electrical stimuli (Figure 4). These artefacts are associated with the transition between no stimulation and the start/end of the sinusoid, resulting in non-linearities that cannot be removed with conventional filtering methods. Further methodological developments are needed to remove these EMG artefacts if muscle activation needs to be quantified within the first ~50ms of a transition to or from painful sinusoidal electrical stimuli. It is important to note that participants reported a decrease in pain even in the absence of changes in load distribution in the unaware condition, although pain modulation occurred earlier in the aware condition. This may have been due to the small whole-body movements associated with standing balance, which could have resulted in pain gating due to the activation of somatosensory afferents but a large gating of pain perception while standing appears unlikely given that others have revealed consistent effects of pain on standing balance (Blouin *et al.*, 2003; Corbeil *et al.*, 2004).

In this study, we described a model to experimentally induce knee pain by using low-frequency sinusoidal electrical stimuli. When using a 4Hz stimulation frequency, the main characteristics of this experimental pain model include: i) a stable perceived pain intensity over a 60s period; ii) EMG signals with minimal stimulation artefacts; iii) task-relevant pain, where the intensity of the stimulation is modulated by a participants' motor behaviour, resulting in correlation between the observed motor adaptation over time and the reported pain ratings. The characteristics of this model allow to overcome several major limitations of currently available experimental pain models, while replicating some of the temporal features of the pain experienced by people with certain musculoskeletal disorders. Low-frequency sinusoidal stimulation provides a novel tool to probe neuromechanical adaptations to task-relevant pain and investigate how physiological signals modulate pain.

COMPETING INTERESTS:

None to declare.

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AUTHOR CONTRIBUTION:

AG and JA contributed equally to this work.

AG, JA and JSB contributed to study design; JA collected the data; AG and JA analyzed the data; AG, JA and JSB interpreted the data; AG, JA and JSB drafted and revised. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Scholars Portal at https://doi.org/10.5683/SP2/YD0AUC

FIGURES:

Figure 1: Experimental setup. Left: illustration of the frontal view of the right knee. Representation of the 64-electrode grid (5 columns by 13 rows) used in the recording of vastus medialis muscle activity. The rectangles represent the electrodes averaged to obtain simulated bipolar recordings in the proximal and distal region of the muscle. The location of the two electrical stimulation electrodes ([+], anode; [-], cathode) on the skin over the infrapatellar fat pad is represented. Right: representative data from a single participant during the task-relevant pain modulation, aware condition. The plot on top shows the change of weight on the right leg in time (black line), expressed as percentage of the weight measured during quiet standing (dashed gray line). The bottom plot shows the amplitude of the signal that was used to drive the intensity stimulation (4Hz sine wave). Stimulation intensities of 4.0 and 6.8 were identified as perceived pain intensities of 0/10 and 3/10 (NRS) before the start of the trial. The stimulation induced a pain of intensity 3/10 NRS when the weight on the right leg was equal or higher than baseline (dark gray panels), a pain of intensity 0/10 when the weight on the right leg was lower than 95% of baseline, and scaled proportionally between 95% and 100% (light gray panels). N=1 representative participant.

Figure 2: Average pain drawing maps. The heatmaps represents the cumulative spatial distribution of the areas reported as painful during stimulation with different waveforms. Dark red pixels (see colormap on the right) identify that the location was reported as painful by 11 participants out of 14.

Figure 3: Habituation to electrical painful stimuli. Pain ratings over time during 60s of painful electrical stimulation induced with different waveforms. Open circles identify individual participants, the black line depicts the median value, and the gray boxes represent the interquartile interval. Significant habituation over time was only observed for the square wave

and the sinusoidal stimulation at 50Hz. The * symbol indicates $p<0.05$ for Generalized Estimating Equations, Friedman tests and Dunn post-hoc tests on N=14 participants.

Figure 4: Effect of waveform on stimulation artefact on the EMG. Left panel: Representation of the EMG raw signals of a representative participant without and during painful electrical stimulations. The EMG signals shown were filtered 20-400Hz. Stimuli were delivered as square waves (top) and 4Hz sinusoids (bottom), both at rest and during a low-force isometric knee extension contraction. Note the large increase in amplitude due to artefacts during stimulation with square waves, but not with the sinusoidal stimulation. Right panel: EMG amplitude spectra for the same signals, before (insets) and after filtering. Note the large peaks introduced by the square wave stimuli. N=1 representative participant.

Figure 5: Pain ratings over time during the aware and unaware conditions and correlation with motor adaptation. Changes in the pain ratings over time are shown for the unaware (left) and aware (middle) conditions. Open circles identify individual participants, the black line depicts the median value, and the gray boxes represent the interquartile interval. Although pain ratings were higher in the unaware than in the aware condition, the change in pain intensity over time was not different between conditions. The scatter plot shows a significant association (Spearman correlation) between weight on the right leg and reported pain intensity (aware condition, 60s). The * symbol indicates $p<0.05$ for Generalized Estimating Equations, Friedman tests and Dunn post-hoc tests on N=12 participants.

Figure 6: Motor adaptation; baseline, unaware and aware conditions. Top panels: change in weight on the right leg over time, expressed as a percentage of the average value at baseline (gray dashed line), for the baseline (left), unaware (middle) and aware condition (right). Bottom panels: weight on the right leg for individual participants (thin lines) and group average (thick lines). Note the small decrease of weight on the right leg at 50s only in the unaware condition,

and the decrease in most participants starting at 20s in the aware condition. The * symbol indicates $p<0.05$ and the # symbol indicates $p=0.08$ for Generalized Estimating Equations, Friedman tests and Dunn post-hoc tests on N=12 participants.

Table 1: Effect of different stimulation waveform, EMG electrode location, filter and task on amplitude of the EMG artefact, measured as change in RMS value measured during painful electrical stimulation compared to baseline. SW: square waves; Prox: proximal electrode; Dist: distal electrode; Contr: during contraction. P values of individual paired Wilcoxon tests are reported on the right column. Bold indicates that RMS amplitude did not increase with the painful electrical stimulation, meaning absence of stimulation artefact. N=12 participants.

Waveform	Location	Filter	Task	Baseline	Stimulation	Difference	P value
SW	Prox	20Hz	Rest	8.3 (6.0-11.8)	325.4 (279.5-380.7)	320.9 (269.9-369.3)	0.003
4Hz	Prox	20Hz	Rest	7.8 (5.9-7.7)	8.6 (6.1-11.6)	**0.8 (0.4-1.3)**	**0.213**
10Hz	Prox	20Hz	Rest	6.4 (5.2-7.7)	7.8 (6.9-11.7)	1.5 (1.3-2.0)	0.003
SW	Dist	20Hz	Rest	6.3 (4.3-6.4)	36.9 (31.3-48-4)	28.7 (24.9-42.9)	0.003
4Hz	Dist	20Hz	Rest	6.3 (4.2-6.7)	7.3 (5.1 – 9.3)	1.1 (0.3-2.0)	0.050
10Hz	Dist	20Hz	Rest	6.2 (3.9-6.5)	9.1 (6.1-13.9)	2.8 (1.7-6.1)	0.003
SW	Prox	20Hz	Contr	31.7 (20.4-32.9)	321.0 (285.7-368.9	297.1 (259.3-342.7)	0.003
4Hz	Prox	20Hz	Contr	30.2 (20.4-36.1)	36.0 (20.9-37.0)	**0.7 (-0.1-2.7)**	**0.131**
10Hz	Prox	20Hz	Contr	31.2 (0.3-34.8)	32.0 (23.0-36.8)	**0.9 (-0.3-4.7)**	**0.075**
SW	Dist	20Hz	Contr	17.9 (12.8-32.5)	49.6 (40.1-57.1)	29.7 (19.3-38.3)	0.003
4Hz	Dist	20Hz	Contr	18.6 (13.6-30.3)	18.8 (15.3-36.5)	**0.8 (-0.9-3.6)**	**0.248**
10Hz	Dist	20Hz	Contr	18.3 (12.7-31.0)	18.5 (15.9-49.3)	2.5 (0.1-8.5)	0.033
SW	Prox	30Hz	Rest	7.5 (5.5-9.5)	325.4 (279.6-380.7)	321.3 (270.9-370.6)	0.003
4Hz	Prox	30Hz	Rest	7.1 (5.4-8.7)	7.5 (5.4-9.9)	**0.5 (0.2-1.2)**	**0.213**
10Hz	Prox	30Hz	Rest	6.2 (4.9-7.0)	7.1 (6.0-9.5)	0.8 (0.7-1.4)	0.003
SW	Dist	30Hz	Rest	6.1 (4.1-6.3)	36.9 (31.3-48.4)	29.5 (24.9-43.1)	0.003
4Hz	Dist	30Hz	Rest	6.2 (4.1-6.5)	6.9 (4.6-7.6)	**0.7 (0.2-0.9)**	**0.091**
10Hz	Dist	30Hz	Rest	6.1 (3.9-6.4)	7.2 (4.9-9.3)	0.8 (0.6-2.6)	0.003
SW	Prox	30Hz	Contr	29.6 (19.0-31.0)	321.0 (285.5-368.7)	297.9 (260.7-344.4)	0.003
4Hz	Prox	30Hz	Contr	28.2 (19.0-34.3)	32.8 (19.3-35.2)	**0.7 (-0.1-1.5)**	**0.182**
10Hz	Prox	30Hz	Contr	30.0 (18.8-32.1)	30.5 (20.8-35.2)	**0.9 (-0.7-3.6)**	**0.155**
SW	Dist	30Hz	Contr	16.5 (11.5-30.9)	48.4 (39.5-56.1)	30.4 (19.8-38.4)	0.003
4Hz	Dist	30Hz	Contr	17.1 (12.8-28.1)	17.4 (13.8-34.0)	**0.5 (-0.8-2.6)**	**0.424**
10Hz	Dist	30Hz	Contr	17.5 (11.6-27.7)	16.8 (12.9-42.9)	**1.5 (0.03-5.1)**	**0.062**

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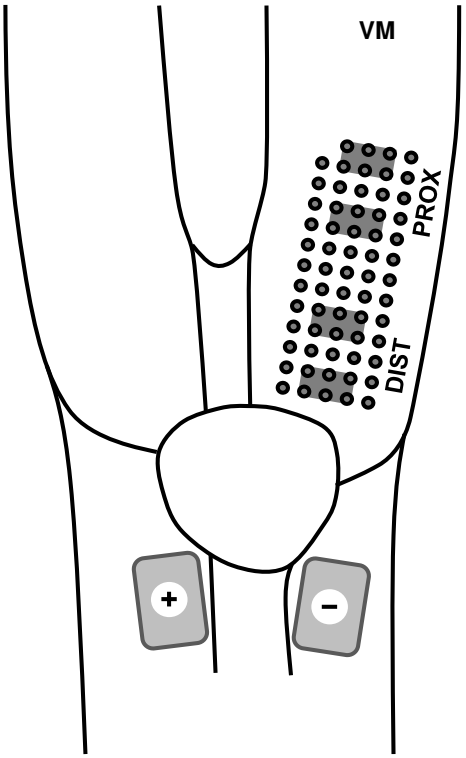
VM
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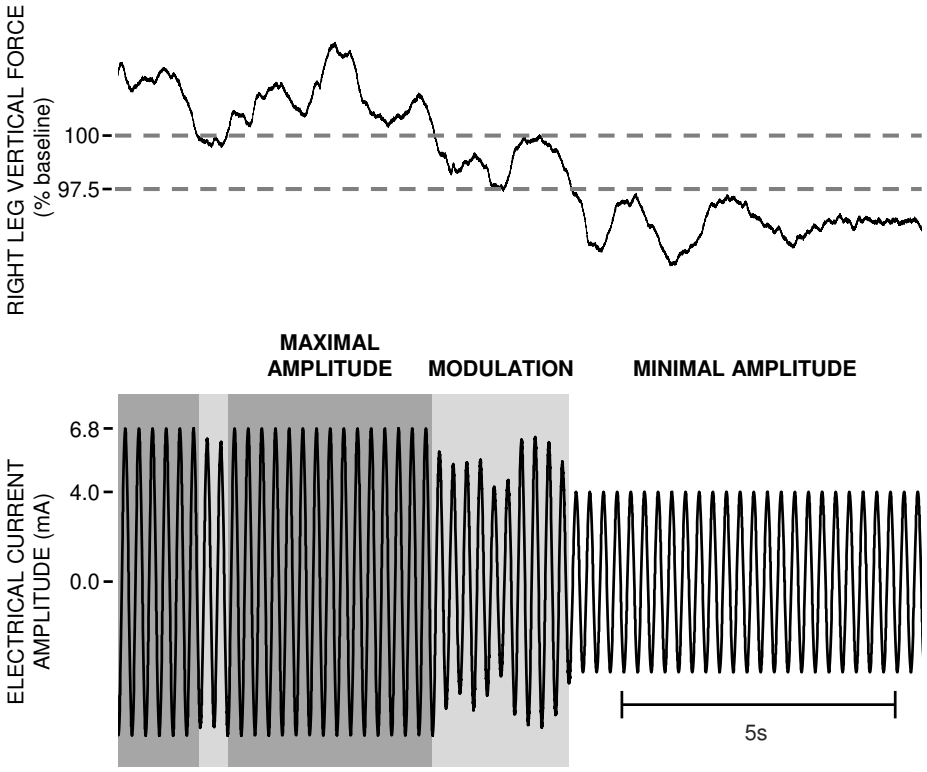
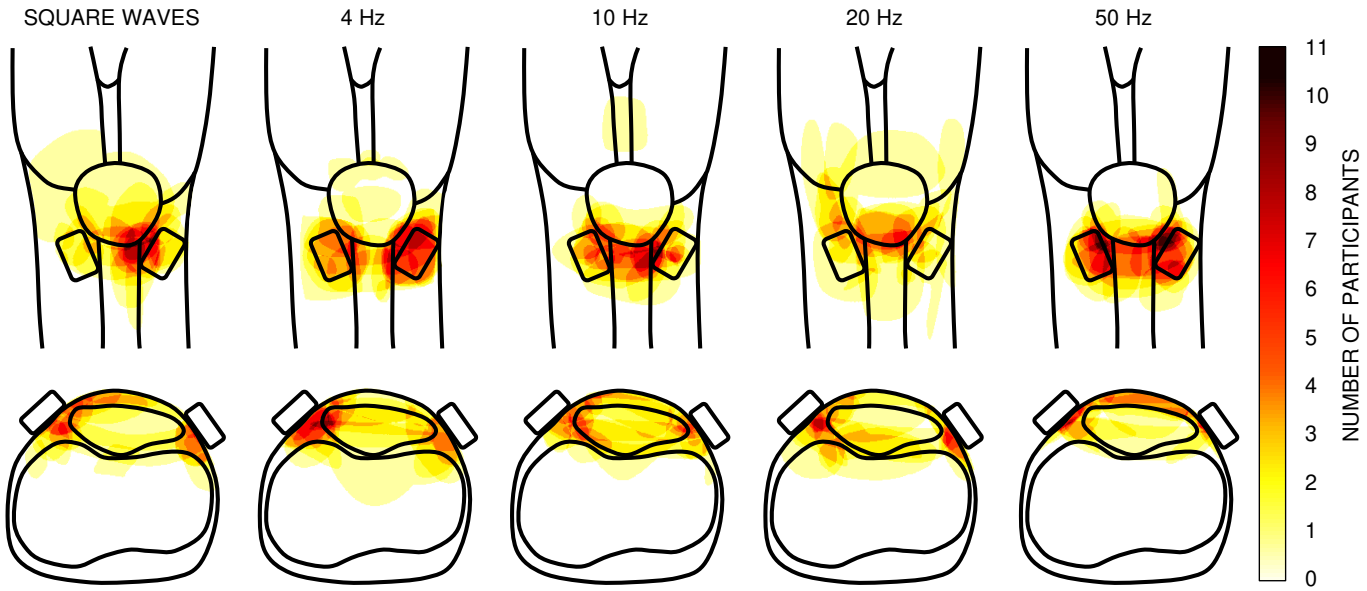
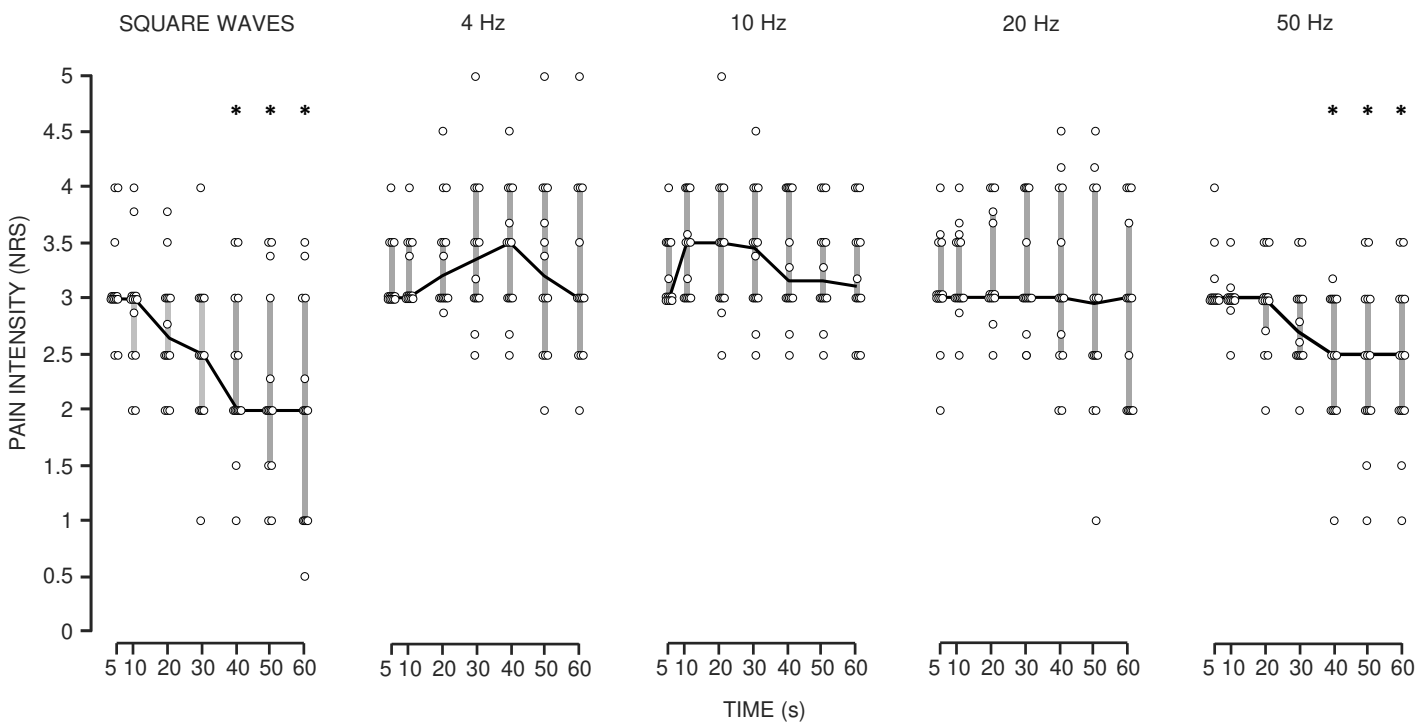
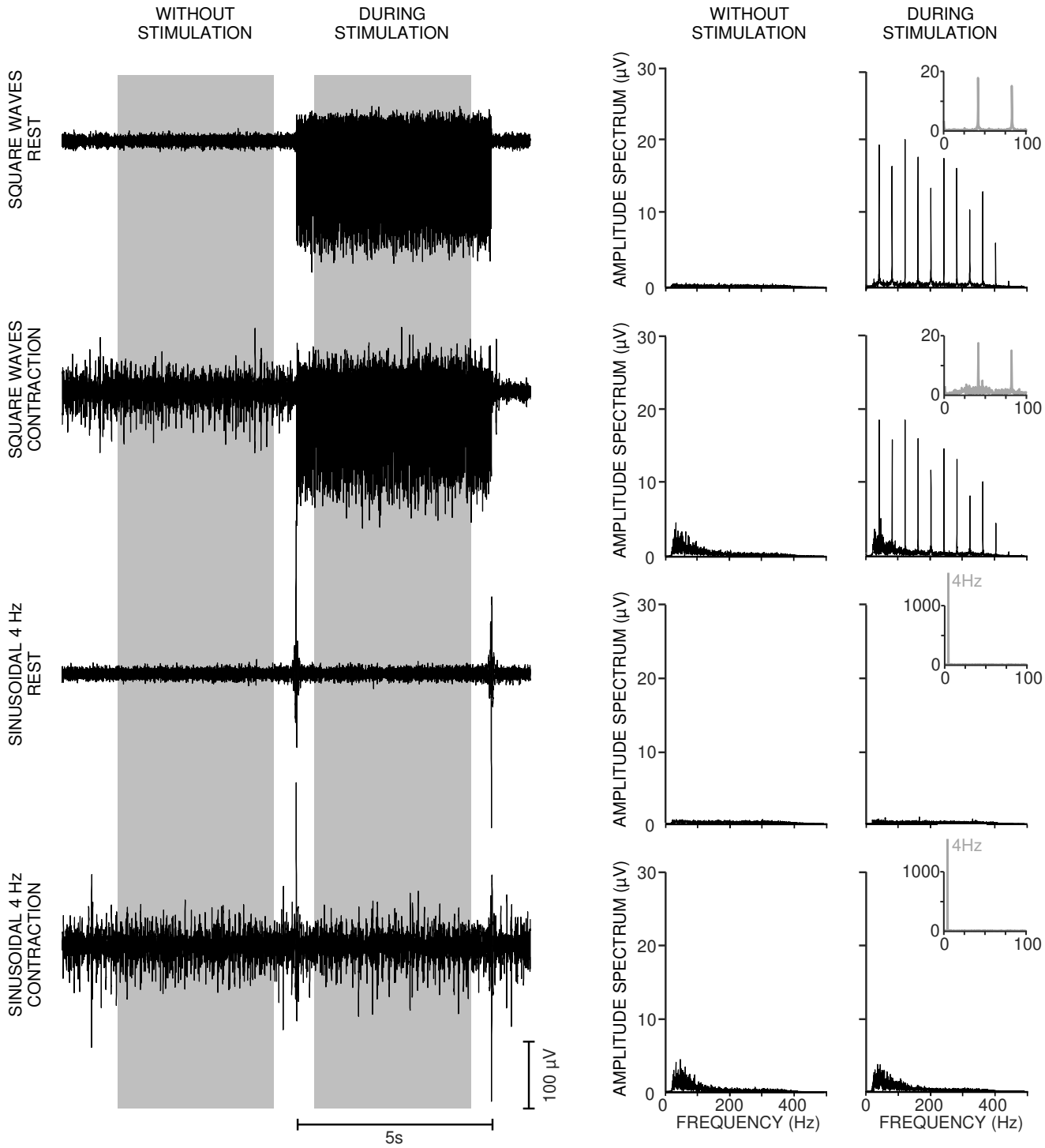
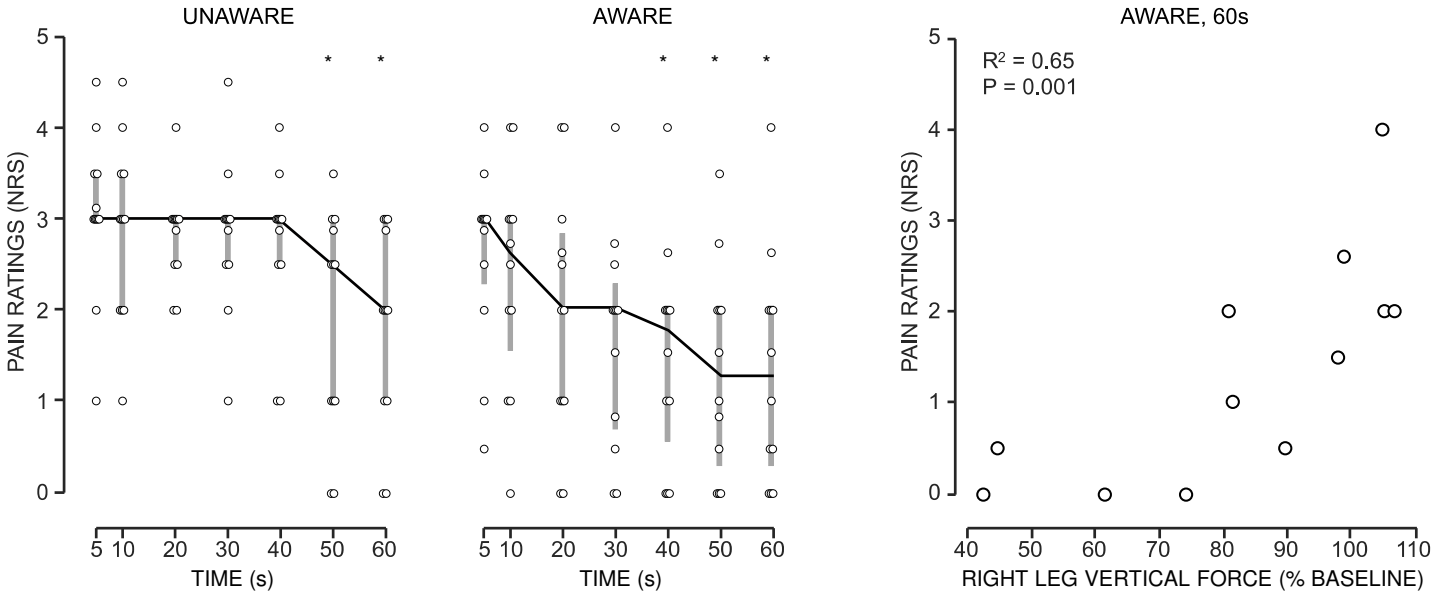
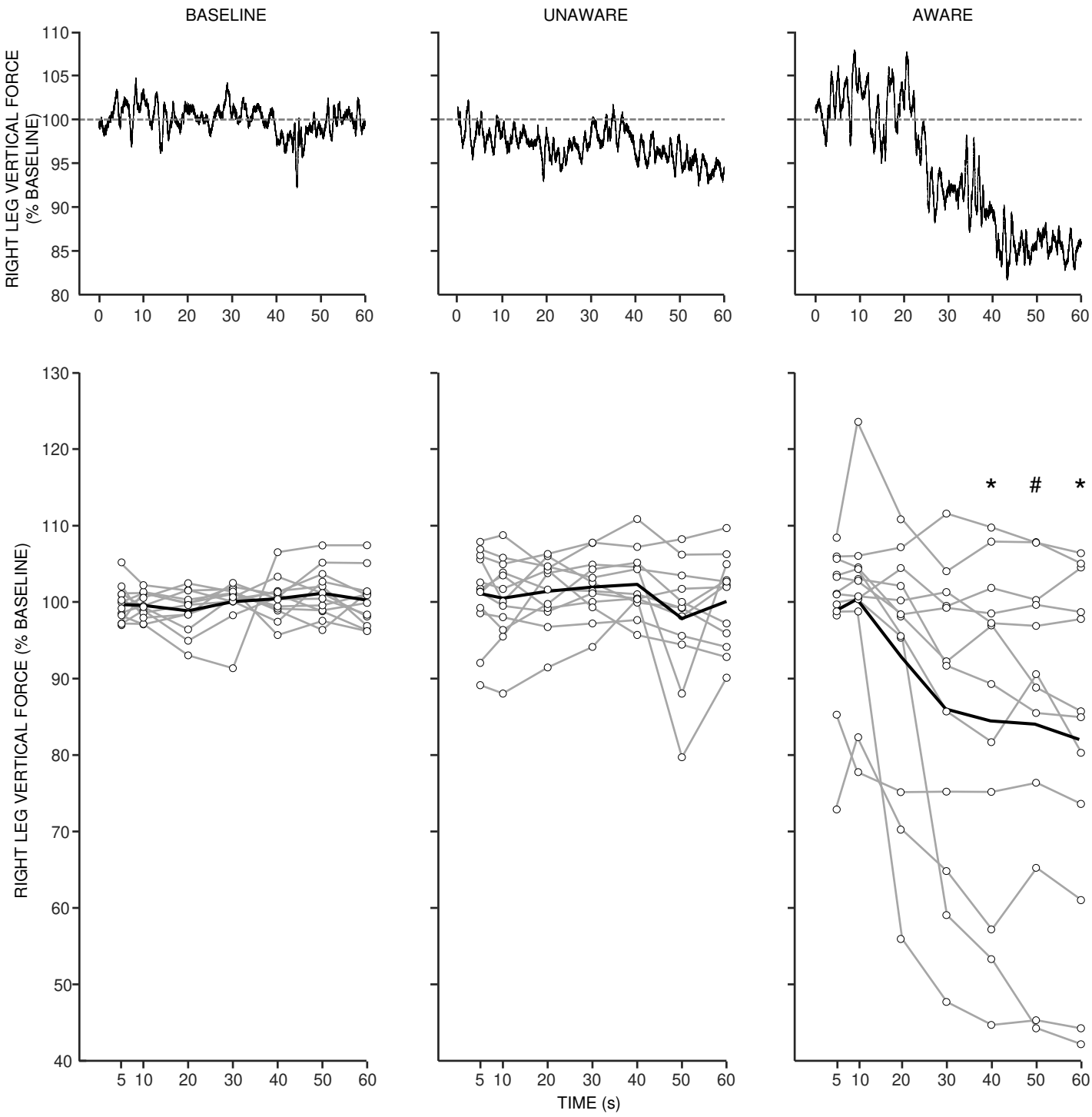

RIGHT LEG VERTICAL FORCE
(% baseline)
100
97.5
MAXIMAL
AMPLITUDE
MODULATION
MINIMAL AMPLITUDE
ELECTRICAL CURRENT
AMPLITUDE (mA)
6.8
4.0
0.0
5s


SQUARE WAVES
4 Hz
10 Hz
20 Hz
50 Hz
11
10
9
8
7
6
5
4
3
2
1
0
NUMBER OF PARTICIPANTS


SQUARE WAVES
4 Hz
10 Hz
20 Hz
50 Hz
PAIN INTENSITY (NRS)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
* * *
* * *
5 10 20 30 40 50 60
TIME (s)


WITHOUT STIMULATION
DURING STIMULATION
SQUARE WAVES REST
SQUARE WAVES CONTRACTION
SINUSOIDAL 4 Hz REST
SINUSOIDAL 4 Hz CONTRACTION
100 µV
5s
WITHOUT STIMULATION
DURING STIMULATION
AMPLITUDE SPECTRUM (µV)
30
20
10
0
4Hz
1000
100
200
400
FREQUENCY (Hz)


UNAWARE
AWARE
AWARE, 60s
PAIN RATINGS (NRS)
TIME (s)
RIGHT LEG VERTICAL FORCE (% BASELINE)
R2 = 0.65
P = 0.001


BASELINE
UNAWARE
AWARE
RIGHT LEG VERTICAL FORCE (% BASELINE)
110
105
100
95
90
85
80
0 10 20 30 40 50 60
130
120
110
100
90
80
70
60
50
40
5 10 20 30 40 50 60
TIME (s)
* # *