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RESEARCH REPORT

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Effects of jaw clenching and mental stress on persistent inward currents estimated by two different methods

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Abstract

Spinal motoneuron firing depends greatly on persistent inward currents (PICs), which in turn are facilitated by the neuromodulators serotonin and noradrenaline. The aim of this study was to determine whether jaw clenching (JC) and mental stress (MS), which may increase neuromodulator release, facilitate PICs in human motoneurons. The paired motor unit (MU) technique was used to estimate PIC contribution to motoneuron firing. Surface electromyograms were collected using a 32-channel matrix on gastrocnemius medialis (GM) during voluntary, ramp, plantar flexor contractions. MU discharges were identified, and delta frequency (ΔF), a measure of recruitment–derecruitment hysteresis, was calculated. Additionally, another technique was used (VibStim) that evokes involuntary contractions that persist after cessation of combined Achilles tendon vibration and triceps surae neuromuscular electrical stimulation. VibStim measures of plantar flexor torque and soleus activity may reflect PIC activation. ΔF was not significantly altered by JC ($p = .679$, $n = 18$, 9 females) or MS ($p = .147$, $n = 14$, 5 females). However, all VibStim variables quantifying involuntary torque and muscle activity during and after vibration cessation were significantly increased in JC ($p < .011$, $n = 20$, 10 females) and some, but not all, increased in MS ($p = .017$–$.05$, $n = 19$, 10 females). JC and MS significantly increased the magnitude of involuntary contractions (VibStim) but had no effect on GM ΔF during voluntary contractions. Effects of increased neuromodulator release on PIC contribution to motoneuron firing might differ between synergists or be context dependent. Based on these data, the background level of voluntary contraction and, hence,

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Abbreviations: ANOVAs, analyses of variance; CIs, confidence intervals; ECG, electrocardiographic; EMG, electromyographic; $\text{EMG}_{\text{sust0.5}}$, root mean square value of the soleus electromyographic signal 0.5 s after vibration; $\text{EMG}_{\text{sust3}}$, root mean square value of the soleus electromyographic signal 3 s after vibration; EMG_{vib}, root mean square value of the soleus electromyographic signal during vibration; GM, gastrocnemius medialis; HD-sEMG, high-density surface electromyographic; JC, jaw clenching; LC, locus coeruleus; MS, mental stress; MU, motor unit; MVC, maximal voluntary contraction; NMES, neuromuscular electrical stimulation; η_p^2, partial eta squared; PICs, persistent inward currents; r_{rm}, repeated measures correlation coefficient; $\text{T}_{\text{sust0.5}}$, self-sustained torque 0.5 s after vibration; T_{sust3}, self-sustained torque 3 s after vibration; T_{vib}, reflexive torque during vibration; VibStim, tendon vibration with superimposed neuromuscular electrical stimulation; ΔF, delta frequency.

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both neuromodulation and ionotropic inputs could influence neuromodulatory PIC enhancement.

KEYWORDS
bistability, HD-EMG, input–output function, motor neuron

1 | INTRODUCTION

Complex physiological processes influence a motoneuron's response to ionotropic input, including the activation of sodium and calcium channels that trigger sustained inward ion flows at motoneuron dendrites (Binder et al., 2020; Heckman, Johnson, et al., 2008). These voltage-dependent channels activate near the motoneuron recruitment threshold (Bennett et al., 1998) and contribute to the initiation, acceleration and maintenance of motoneuron firing. Magnitudes of the ensuing persistent inward sodium or calcium currents (PICs) and their effects on synaptic input-firing rate non-linearities vary with levels of the monoamines serotonin and noradrenaline, which are released onto the motoneurons (Lee & Heckman, 2000). Thus, understanding how monoaminergic drive modulates PIC contribution to motoneuron firing may offer insight into motoneuron function in healthy (Trajano et al., 2020), older (Hassan et al., 2021; Orssatto, Borg, et al., 2021; Orssatto, Mesquita, & Phillips, 2021) or diseased (D'Amico et al., 2013) populations.

Jaw clenching is one non-pharmacological intervention that might enhance PIC activity. Although underlying mechanisms remain unclear, several studies have shown improvements in motor performance during jaw clenching (Ebben, 2006; Miró et al., 2021). These improvements could be related to PIC facilitation. Motor activity likely increases serotonin release from the raphe nuclei onto the motoneurons (Alvarez et al., 1998; Bowker et al., 1981; Jacobs et al., 2002; Kawashima, 2017; Veasey et al., 1995), and serotonergic neurons project diffusely onto spinal motor pools (Skagerberg & Björklund, 1985). Thus, voluntary contraction of one muscle should theoretically lead to serotonergic enhancement of PICs in another muscle (Wei et al., 2014). This could also explain previous findings of greater Hoffman reflexes and lower reciprocal inhibition of the plantar flexors during jaw clenching (Hirabayashi et al., 2021; Takada et al., 2000) and seminal observations of facilitation of reflexes during Jendrassik manoeuvres (Jendrássik, 1885). Another potential PIC-enhancing, non-pharmacological intervention is mental stress. The locus coeruleus (LC) provides noradrenergic innervation to spinal motoneurons (Proudfit & Clark, 1991), with increased activity in response to stress and arousal (Ross & Van Bockstaele, 2021; Valentino & Van Bockstaele, 2008). Accordingly, stressful mental calculation has led to changes in motor output that could be caused by PIC facilitation (Ji et al., 2016; Pereira et al., 2018; Yoon et al., 2009). Whilst this PIC facilitation could be beneficial in some conditions (e.g., fight or flight responses to threat [Jansen et al., 1995] or to override inhibitory signals whilst exercising in the heat [Watson et al., 2005]), it could be detrimental in others (e.g., spasticity after traumatic brain injury [Tsuda et al., 2020]).

PICs cannot be directly measured in humans. However, some aspects of motor unit (MU) firing can act as markers of PIC presence. Firing rate hysteresis, observed as MU derecruitment at a lower synaptic input level than MU recruitment, is a hallmark of PIC effects in human MU firing. During ramp contractions, the firing rate of one MU is used to estimate differences in net synaptic input at recruitment and derecruitment of another MU (paired MU technique—Gorassini et al., 1998, 2002a, 2002b). This change in frequency (delta frequency [ΔF]) provides an estimate of the PIC contribution to MU firing, as validated in animal models (Bennett, Li, Harvey, & Gorassini, 2001) and computer simulations (Powers & Heckman, 2015). Nonetheless, methodological limitations include the inability to estimate PIC-induced effects without voluntary drive and the relatively time-consuming analysis required after intramuscular (Foley & Kalmar, 2019; Marchand-Pauvert et al., 2019; Wilson et al., 2015) or high-density surface-based (Hassan et al., 2021; Khurram et al., 2021; Orssatto, Mackay, et al., 2021) electromyogram recording.

A proposed alternative, hereafter referred to as VibStim, combines tendon vibration and bursts of neuromuscular electrical stimulation (NMES) in an inactive muscle (Bochkezanian et al., 2018; Espeit et al., 2021; Kirk et al., 2019; Magalhães & Kohn, 2010; Mesquita et al., 2021, 2022; Trajano et al., 2014), producing involuntary muscle contractions with amplitudes and patterns that could theoretically result from PIC activation (Mesquita et al., 2021). During a VibStim trial, a progressive increase in involuntary torque aligns with calcium-dependent facilitation of PICs (Svirskis &

Hounsgaard, 1997). A self-sustained muscle force after cessation of vibration and NMES is consistent with the phenomenon of PIC-related bistability (Lee & Heckman, 1998). The influence of muscle length (Trajano et al., 2014) is also consistent with direct measurements of PIC amplitude (Hyngstrom et al., 2007). Moreover, reductions in both soleus ΔF values and involuntary plantar flexion torque have been found after muscle stretching (Trajano et al., 2014, 2020).

We previously found reductions in ΔF during whole-body relaxation and reciprocal inhibition. However, we observed a decline in some, but not all, VibStim variables (Mesquita et al., 2022). This lack of consistency might indicate that VibStim is not sufficiently sensitive to detect PIC changes, at least under some physiological conditions. This discrepancy might also be partly explained by a 'floor effect', given the very low levels or the absence of involuntary sustained torque in many participants in the VibStim control condition. Thus, comparing PIC estimates from both techniques during interventions that should facilitate PICs is also of interest because the estimates should not be limited by floor effects.

The present study examined the effects of both jaw clenching and mental stress on (1) the PIC contribution to plantar flexor motoneuron firing, estimated using the paired MU technique, and (2) the magnitude of involuntary plantar flexion torque and muscle activity assessed using VibStim, which may reflect PIC activation. An exploratory analysis was also completed to compare effects between males and females. It was hypothesised that PICs estimated by both techniques would increase in both jaw clenching and mental stress. It was also hypothesised that there would be no differences between males and females in the contribution of PICs to motoneuron firing.

2 | METHODS

This research formed part of a larger study assessing the acute effects of multiple interventions on the contribution of PIC activity to MU firing in plantar flexor motoneurons (using the paired MU technique) and on the magnitude of ongoing, involuntary plantar flexion torque and muscle activity during application of tendon vibration with NMES (VibStim). Data relating to the effects of interventions in which we hypothesised a decrease in PIC activity (reciprocal inhibition and whole-body relaxation) have been previously reported (Mesquita et al., 2022), whilst data describing the effects of interventions in which an increase in PIC activity was expected (jaw clenching [JC] and mental stress [MS]) are presented herein.

2.1 | Participants and ethical approval

Twenty-one healthy adults aged 19–36 years (11 males and 10 females; age: 26.3 ± 5.1 years; body mass: 79.4 ± 15.6 kg; height: 172.9 ± 10.1 cm) volunteered for this study. Exclusion criteria included current or recent injuries (recovered for less than 6 months), medications that could influence the monoaminergic system, and diagnosed neurological disorders. The procedures were approved by the Human Research Ethics Committee of Edith Cowan University (reference number: 22306) and performed according to the Declaration of Helsinki, except for registration in a database. Participants were fully informed of any risks or discomforts associated with the procedures before giving their written informed consent to participate.

2.2 | Procedures

The participants visited the laboratory on three separate occasions at the same time of the day. In the first visit, participants were familiarised with maximal plantar flexion strength assessment, the VibStim protocol and the ramp contractions used during the paired MU recordings. Participants returned to the laboratory for two experimental sessions separated by at least 24 h (mean = 3.0 ± 1.9 days apart) and were asked to refrain from caffeinated foods and beverages within 12 h prior to the sessions.

In all sessions, participants were seated in the chair of a dynamometer (Biodex System 4, Biodex Medical System, USA), with the hips at 50° of flexion (0° = extended neutral position), right knee fully extended (0°) and right ankle dorsiflexed at 10° (0° = anatomical position). The foot was firmly fixed to the plantar flexor attachment, and the axis of rotation was aligned with the lateral malleolus.

On each experimental day, participants underwent two experimental conditions with VibStim and then the same two conditions with the paired MU technique. The four conditions were performed in a randomised order over two experimental days with two conditions per day. Only two of the conditions are reported here (see Mesquita et al., 2022). As pilot testing revealed that skin preparation for high-density surface electromyographic (HD-sEMG) recordings increased participant discomfort during NMES, VibStim was always performed first.

Before testing, NMES electrodes (self-adhesive; 5 × 9 cm; Dura-Stick Plus, DJO Global, USA) were placed over the triceps surae muscles with the cathode placed transversely and distal to the popliteal crease and the anode transversely over the distal gastrocnemius–

Achilles muscle–tendon junction. Bipolar electrodes to measure electromyographic (EMG) signals were also placed over soleus. As a warm-up, participants performed four submaximal isometric plantar flexion contractions (∼3-s contractions at 20%, 40%, 60% and 80% of perceived maximal effort) and then three maximal voluntary contractions (MVCs), interspersed by 90 s of rest. After 3 min of rest, a constant-current stimulator (DS7, Digitimer Ltd, UK) was used to deliver 0.5-s 20-Hz trains of NMES (1-ms pulse width) to the plantar flexors at increasing intensities to identify the current required to evoke 20% of MVC torque (the maximum value achieved during the familiarisation session was used).

Control trials were always performed before the experimental trials (i.e., for each condition: 3 control + 3 experimental trials using the paired MU technique and 2 control + 2 experimental trials using VibStim). There were 90-s rest periods between trials, and ∼20 min separated tests using the different techniques (between VibStim and paired MU techniques), allowing equipment preparation, skin preparation and HD-sEMG electrode placement, as described below. After the first experimental session, electrode locations were marked on the skin with a permanent marker to ensure consistent between-session electrode placement.

2.3 | Paired motor unit technique

Real-time visual feedback of plantar flexion torque was presented on a large screen (ISO-AUXSE adapter and OT BioLab+ software, OT Bioelettronica, Italy), and participants were instructed to follow a torque path to perform isometric, ramp, plantar flexion contractions to 20% of MVC (assessed during the familiarisation session). Contraction duration was set to 20 s, with an 8-s ascending phase, 4-s hold phase and 8-s descending phase, producing rates of contraction/relaxation of 2.5% MVC/s.

A flexible adhesive grid of HD-sEMG electrodes (GR10MM0804, 10-mm inter-electrode distance; OT Bioelettronica, Italy) was used to record from 32 sites on gastrocnemius medialis (GM). Before electrode placement, the skin was shaved, abraded with sandpaper and swabbed with alcohol. Then, the grids were attached to the skin (distal region of GM) by bi-adhesive foam with skin-to-electrode contact optimised by filling the adhesive foam wells with conductive cream (AC Cream, Spes Medica, Italy). A damp strap electrode (WS2, OT Bioelettronica, Italy) around the ankle was used as a reference electrode. A Sessantaquattro system (OT Bioelettronica, Italy) was used to amplify EMG signals (256×), with a band-pass filter set to 10–500 Hz, sampled at 2000 Hz in monopolar mode, digitally converted (16-bit resolution) and then transferred to a personal computer. The OT BioLab+ software (Version 1.3.0.0) was used to record and visually inspect EMG signals throughout the protocol.

2.3.1 | Data analysis—Paired motor unit technique

The PIC contribution to motoneuron firing was estimated with the paired MU technique (Gorassini et al., 1998, 2002a, 2002b), where the smoothed firing rate of a lower threshold MU (control unit) was used as an estimate of net synaptic input to a higher threshold MU (test unit). The difference between the smoothed firing rates of the control unit when the test unit was derecruited was subtracted from the control unit rate when the test unit was recruited to obtain a ΔF (change in frequency) value. This value is then an estimation of PIC contribution to motoneuron firing of the test unit (Figure 1b).

HD-sEMG decomposition, MU tracking, careful visual inspection of spike trains, and the smoothing process of instantaneous firing rates were conducted as previously described (Mesquita et al., 2022). First, the HD-sEMG signals were notch-filtered in order to remove the line interference and high-pass filtered with a zero-phase second-order filter to emphasise differences between MU action potential shapes belonging to different MUs. Then, the filtered HD-sEMG signals were decomposed using the Convolution Kernel Compensation method (Holobar & Zazula, 2007), with the number of decomposition runs set to 50. The identified MUs were manually inspected in DEMUSE tool (University of Maribor, Slovenia) by an experienced operator to assess the accuracy of MU identification, including manual editing of the spike trains. In cases where fewer than 10 were accurately detected, the HD-sEMG signals from a single ramp were subjected to a further round of 50 decomposition runs (redecompose function in DEMUSE tool). Assuming that the lower yield of MUs was due to low signal-to-noise ratios, this second round of decomposition runs did not involve the use of notch and high-pass differential filters. Not utilising a notch filter allowed decomposition of the full range of the processed signals, and the high-pass filter was not used as this may further decrease signal-to-noise ratios. MUs identified in this second run were combined with those from the first run, removing the potential duplicates of MUs identified in both decomposition runs. The MU filters of the additional MUs were manually optimised using the DEMUSE tool and then applied to other trials for MU tracking purposes, as previously described (Mesquita et al., 2022). MU firing was preferentially fitted with a fifth-order

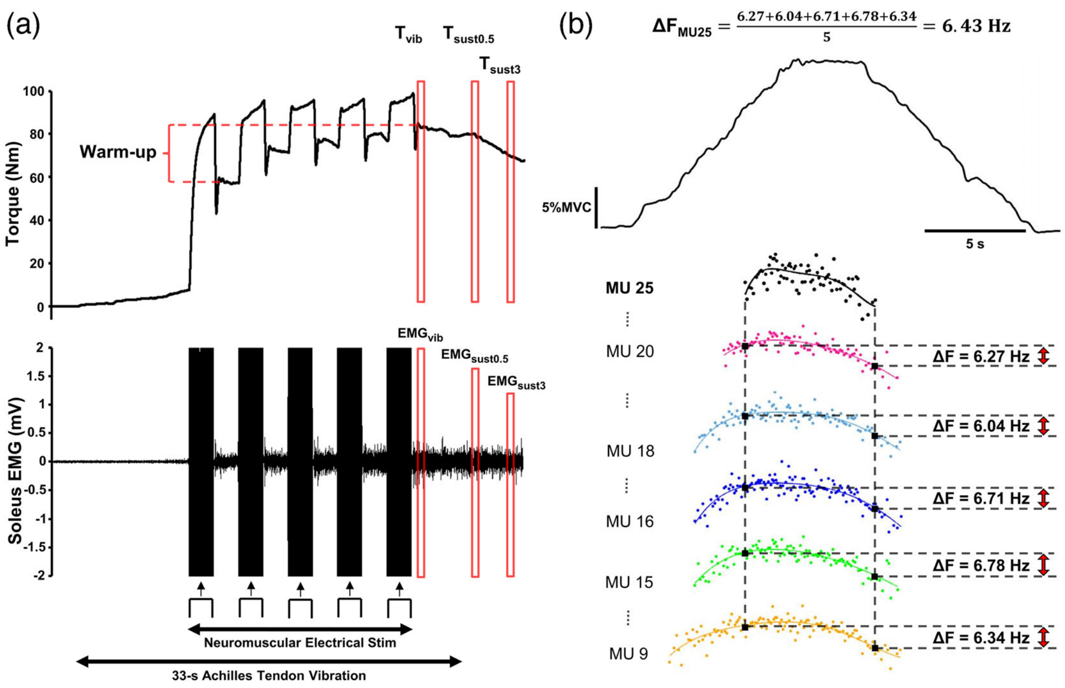

FIGURE 1 Data from one participant during single trials using each technique. (a) Plantar flexion torque and soleus electromyographic (EMG) response to tendon vibration with superimposed neuromuscular electrical stimulation (VibStim). The Achilles tendon was mechanically vibrated for 33 s, with neuromuscular electrical stimulation (NMES) applied after 10 s of vibration whilst tendon vibration continued. $\text{EMG}_{\text{sust0.5}}$ and $\text{EMG}_{\text{sust3}}$, root mean square value of the soleus EMG signal 0.5 and 3 s after vibration, respectively; EMG_{vib}, root mean square value of the soleus EMG signal during vibration; $T_{\text{sust0.5}}$ and T_{sust3}, self-sustained torque 0.5 and 3 s after vibration, respectively; T_{vib}, reflexive torque during vibration; warm-up, difference between T_{vib} and torque after the first burst of NMES. (b) Calculation of delta frequency (ΔF) from one test unit identified during a ramp contraction in a trial for the paired motor unit (MU) technique. Torque trace (black, at the top) is shown during a 20-s ramp contraction (8-s ascending phase, 4-s hold phase and 8-s descending phase) up to 20% of the torque reached in a maximal voluntary contraction (MVC). The instantaneous and smoothed (polynomial fit) firing rates of 6 out of 25 MUs that were identified in this trial are shown to demonstrate the paired MU analysis approach. MU 25 (black) was the test unit, which formed suitable pairs with five other control units. Thus, ΔF of MU 25 (ΔF_{MU25}) was the average of ΔF scores computed when paired with those five control units. Dashed vertical lines mark the time of occurrence of recruitment and derecruitment of the test unit. Dashed horizontal lines indicate the smoothed firing frequency of the control units at these times, with the magnitude of difference between these two (ΔF) marked by the red arrows.

polynomial function. A fourth-order polynomial was used when edge effects were identified at MU recruitment or derecruitment (i.e., mismatch between the change in the instantaneous and smoothed firing rate) upon visual inspection of the polynomials. MUs were not included for further analysis if edge effects were observed for both fifth- and fourth-order polynomials. A MU pair was only considered for analysis if the test unit was derecruited before the control unit. Furthermore, to ensure that the control unit was a suitable estimate of the net synaptic input, several criteria were adopted: test units had to be recruited at least 1.0 s after the control units (Hassan et al., 2020); rate-to-rate correlations between the smoothed firing rate polynomials of the test and control units had to be $r \geq .7$ (Stephenson & Maluf, 2011), with the first 500 ms of the test unit being excluded from the correlation analysis (Mottram et al., 2009); and the control unit had to increase its firing frequency by at least .5 Hz after the recruitment of test unit (Stephenson & Maluf, 2011).

A two-ramp, multi-control, repeated measures approach was used to estimate the contribution of PIC activity to the firing of test units (Mesquita et al., 2022). First, ΔF values were computed for all possible MU pairs within each ramp. Second, one combined set of pairs was determined from the two control ramps and a second set from the two experimental ramps. If a specific pair was present in both ramps (i.e., Control 1 and Control 2 or Experimental 1 and Experimental 2), the average ΔF was computed. Third, only the MU pairs that were identified in both control and experimental ramps were considered for further analysis. Finally, ΔFs from individual test units were calculated as the average value obtained when the units were paired with multiple suitable control units, as previously conducted (Trajano et al., 2020). This approach reduces intra-participant ΔF variability and ensures the attribution of a single estimation of the magnitude of PIC contribution to motoneuron firing of each test unit. LabChart macros (Version 8.1.16, ADInstruments, New Zealand) were used to quantify onset and offset parameters. The identification of suitable pairs and calculation of ΔF values were conducted in Excel (Version 2106, Microsoft Corporation, USA).

2.4 | VibStim

During VibStim trials, NMES bursts were applied during ongoing tendon vibration to evoke involuntary muscle contractions (Mesquita et al., 2021; Trajano et al., 2014). In each trial, a hand-held vibrator (Vibrasens, Techno Concepts, France) applied with steady pressure on the posterior aspect of the Achilles tendon delivered vibration for 33 s at 115 Hz and 1-mm amplitude. Ten seconds after vibration onset, 5 × 2-s NMES monophasic rectangular bursts at 4-s intervals (2-s on and 2-s off) with wide (1-ms) pulse width, 20-Hz frequency and intensity that evoked 20% of MVC torque (current intensity range: 8.4–61.4 mA) were applied to the triceps surae whilst tendon vibration continued. We have previously found that this combination of electrical parameters in VibStim trials provides the most robust increases in evoked involuntary plantarflexion torque (Mesquita et al., 2021). In each experimental session, the current required to evoke 20% of MVC torque was re-assessed. During trials, participants were instructed to hold shoulder straps of the chair, to look forward at the blank display monitor and to not voluntarily activate their leg muscles.

EMG signals from soleus were digitised at 2000 Hz (PowerLab 16/30, ADInstruments, New Zealand), band-pass filtered (10–1000 Hz), and amplified (BioAmp EMG system, ADInstruments, New Zealand), using LabChart software (Version 8.1.16, ADInstruments, New Zealand). Bipolar surface electrodes (BlueSensor N-00-S, 28 mm^2, Ambu, Denmark) were placed below the gastrocnemius over the mid-dorsal line of the posterior shank, with 1-cm inter-electrode distance and the reference electrode on the lateral malleolus. The skin under the electrodes was shaved, abraded with sandpaper, and swabbed with alcohol.

2.4.1 | Data analysis—VibStim

Four torque and three EMG variables were calculated (Figure 1a) using LabChart macros (Version 8.1.16, ADInstruments, New Zealand). Torque-related variables are presented as % MVC, and soleus EMG amplitude was quantified as the root mean square (rmsEMG) value in the 500-ms windows of interest (Figure 1a). A digital low-pass filter (cut-off frequency of 8 Hz, determined by residual analysis) was applied to the torque data, and EMG data were filtered with a digital band-pass filter (20–500 Hz).

The following variables were calculated:

1. Reflexive torque during vibration (T_{vib}) and soleus EMG during vibration (EMG_{vib}): mean torque and rmsEMG in a 500-ms window commencing 500 ms after the cessation of NMES, but during tendon vibration.
2. Self-sustained torque 0.5 and 3 s after vibration ($T_{sust0.5}$ and T_{sust3}) and soleus EMG at the same times ($EMG_{sust0.5}$ and EMG_{sust3}): mean torque in 500-ms windows commencing 500 ms and 3 s after the cessation of tendon vibration and rmsEMG over the same time windows.
3. Warm-up: the difference between T_{vib} and the mean torque in a 500-ms window commencing 500 ms after the first burst of NMES.

2.5 | Interventions (conditions)

2.5.1 | Jaw clenching

Participants were asked to clench their teeth on a plastic mouthguard (Howies Hockey Tape, USA) as forcefully as possible for 10 s before the trials and during the trials. These mouthguards were self-adapting and were fitted through a 'boil-and-bite' process. At the start of the session on the day in which JC was performed, the mouthguard was submerged in boiling water for a short period of time and then participants were asked to bite down on the heated plastic to mould it around their teeth.

2.5.2 | Mental stress

Participants were asked to count down from a random number between 200 and 1000 in intervals of 13, as fast as possible (adapted from Noteboom et al., 2001). The random numbers were communicated to the participants by a research assistant who also generated a loud sound through the speakers, similar to a game show, when the participant gave a wrong answer or took more than ∼2 s to give an answer. A new random number was communicated to the participant after each wrong answer. This task started 30 s before the trials and continued during the trials.

2.6 | Manipulation check

Immediately after each trial, self-rated stress was measured. Participants answered the question 'Please indicate how relaxed or stressed you felt during the trial'. Participants indicated their answer on a 7-point Likert scale, where 1 = *Very relaxed*, 2 = *Relaxed*, 3 = *Somewhat relaxed*, 4 = *Neither relaxed nor stressed*, 5 = *Somewhat stressed*, 6 = *Stressed* and 7 = *Very stressed*. Researchers were blinded to the participants' answers.

Electrocardiographic (ECG) data were recorded during VibStim trials via electrodes (BlueSensor N-00-S, 28 mm^2, Ambu, Denmark) placed on the left lower ribcage and below the left clavicle with a reference electrode on the dorsum of the left hand. The signal was band-pass filtered (10–120 Hz) and digitised at 2000 Hz (PowerLab 16/30, ADInstruments, New Zealand) to measure heart rate offline. Pilot testing demonstrated that ECG electrode connection increased the background noise in HD-sEMG signals, and thus, ECG data were not recorded during ramp contractions. The heart rate variability module in LabChart was used to analyse ECG data, automatically detecting R-wave peaks.

2.7 | Statistical analysis

Most statistical analyses of MU variables were conducted in R (Version 4.0.4) using RStudio environment (Version 1.4.1106), except where indicated. The effect of each experimental intervention on ΔF of all test units and on MU recruitment threshold (i.e., torque at the times when the MU began discharging action potentials), MU derecruitment threshold (i.e., torque at the times when the MU stopped discharging action potentials), and peak smoothed firing rate of the whole sample of MUs was examined using separate repeated measures nested linear mixed-effects models (*lmerTest* package; Boccia et al., 2019; Kuznetsova et al., 2017). Only MUs that could be tracked between control and experimental trials were included in statistical analyses. A random intercepts (parallel slopes) model was used with 'condition', 'sex' and their interaction as fixed effects and 'participant' and 'test unit' (or 'MU') as random effects. Residuals were plotted against fitted values to assess whether variance was consistent across the fitted range, and Q–Q plot inspection was used to assess the assumption of normality of residuals. Estimated marginal means (with 95% confidence intervals [CIs]) and their differences (with 95% CIs) were determined in post hoc tests (*emmeans* package; Lenth & Lenth, 2018). For visualisation purposes, kernel density estimations (density curves) of the MU variables were plotted (*gghalves* package; Tiedemann, 2020), as a depiction of data distribution. These density curves are a smooth empirical probability density function, and each data point has an equivalent influence on the final distribution. Given the non-independent nature of the observations, repeated measures correlation coefficients of MU data were computed (*rmcorr* package; Bakdash & Marusich, 2017). The smoothed firing rate of the test and control units were plotted (2000 data points per second), and the rate-to-rate Pearson's correlation coefficients were computed in Excel (Version 2106, Microsoft Corporation, USA). An overall comparison between the number of identified MUs of males and females was conducted with a random intercepts (parallel slopes) model with 'sex' as a fixed effect and 'participant' as a random effect, and independent two-tailed *t* tests were used for comparisons within each intervention. Differences in mean absolute error in ramp performance were examined with paired two-tailed *t* tests.

Statistical analyses of dependent variables obtained during VibStim tests were conducted in SPSS Statistics software (Version 27, SPSS Inc., USA). Data normality was examined using the Shapiro–Wilk test. Torque and EMG variables were averaged across the two trials in each condition and subjected to separate 2 (sex) × 2 (condition: control vs. experimental) mixed analyses of variance (ANOVAs). The least significant difference method was used for post hoc comparisons, with effect sizes reported as partial eta squared ($\eta_p{}^2$). An exploratory analysis was also conducted to examine differences with the exclusion of participants who did not exhibit an involuntary evoked torque in the time window of interest during control (i.e., torque <1% MVC). Paired *t* tests were conducted when differences between experimental and control were normally distributed, or alternatively, Wilcoxon signed-rank tests were performed.

Additional exploratory Pearson and Spearman's correlations were computed in SPSS Statistics software (Version 27, SPSS Inc., USA). Finally, heart rate and self-rated scores were subjected to separate 2 (sex) × 2 (condition: control vs. experimental) mixed ANOVAs. Statistical significance was set at an alpha level of .05 in all statistical analyses.

3 | RESULTS

3.1 | Summary

The main results of the study are summarised in Table 1. The estimate of the contribution of PICs to motoneuron firing (ΔF) using the paired MU technique remained unaltered in both JC and MS. In VibStim, all variables

TABLE 1 Summary of main results.

Variable	JC	MS
ΔF	↔	↔
Recruitment threshold	↓	↓
Derecruitment threshold	↑♀, ↓♂	↑♀, ↓♂
Peak smoothed firing rate	↑	↑
T_{vib}	↑	↑
EMG_{vib}	↑	↑
$T_{sust0.5}$	↑	↑, EDA: ↔
$EMG_{sust0.5}$	↑	↔
T_{sust3}	↑	↔
EMG_{sust3}	↑	↔ ♀, ↑ ♂
Warm-up	↑	↑

Note: Variables quantified during the paired motor unit technique trials are in the upper section of the table, and those quantified during the VibStim trials are in the lower section of the table. '↓' indicates significant decrease from control to experimental trials. '↑' indicates significant increase from control to experimental trials. '↔' indicates no significant change between control and experimental trials.

Abbreviations: EDA, exploratory data analysis, excluding participants who did not exhibit an involuntary evoked torque in the time window of interest during control; $EMG_{sust0.5}$, root mean square value of the soleus electromyographic signal 0.5 s after vibration; EMG_{sust3}, root mean square value of the soleus electromyographic signal 3 s after vibration; EMG_{vib}, root mean square value of the soleus electromyographic signal during vibration; JC, jaw clenching; MS, mental stress; $T_{sust0.5}$, self-sustained torque 0.5 s after vibration; T_{sust3}, self-sustained torque 3 s after vibration; T_{vib}, reflexive torque during vibration; warm-up, torque increase during the trial; ΔF, delta frequency.

TABLE 2 Descriptive statistics for motor unit data across participants.

	Jaw clenching	Mental stress
Decomposed MUs	236	193
% polynomials fifth degree	89.3	89.2
% polynomials fourth degree	11.1	10.8
% polynomials excluded	4.4	4.6
MUs included for analysis	212	186
Test units	130	100
Pairs	628	464
Participants with MUs for analysis (F/M)	18 (9/9)	17 (7/10)
Participants with test units for analysis (F/M)	18 (9/9)	14 (5/9)

Note: 'Decomposed MUs' indicates number of accurate motor units identified and manually inspected. '% polynomials fifth/fourth degree' indicates percentage of MUs fitted with a fifth- or fourth-order polynomial function across contractions. '% polynomials excluded' indicates percentage of MUs that could not be fitted with a fifth- or fourth-order polynomial across contractions. 'MUs included for analysis' indicates number of motor units with a polynomial fit that could be tracked from control to experimental trials. 'Test units' indicates number of test units used for analysis and that could be tracked from control to experimental trials. 'Pairs' indicates number of pairs that could be tracked from control to experimental trials. 'Participants with MUs for analysis (F/M)' indicates number of participants included in recruitment threshold, derecruitment threshold and smoothed peak firing rate (females/males) analyses. 'Participants with test units for analysis (F/M)' indicates number of participants included in delta frequency (ΔF) analysis (females/males).

were significantly increased in JC, whilst some increased in MS.

3.2 | Descriptive motor unit data

Across the different ramps, 820 spike trains were discriminated in JC from HD-sEMG recorded from GM whilst 526 spike trains were discriminated in MS. The proportion of MUs that were tracked between control and experimental trials was 89.9% in JC and 92.0% in MS. The average number of control units that were paired with test units was 5 for both interventions (range in JC: 1–14; range in MS: 1–10). Overall, whilst concurrently taking both interventions into consideration, there was no evidence of a significant difference in the number of identified MUs between males and females ($p = .068$). However, males had a significantly higher number of MUs (16.1 ± 5.4 vs. 7.6 ± 6.9, $p = .010$) and test units (10.6 ± 4.4 vs. 3.9 ± 5.6, $p = .013$) that were used in statistical analyses per participant in JC. In MS, there was no difference in the number of MUs (males: 13.2 ± 8.0 and females: 7.7 ± 6.1, $p = .147$) or test units (males: 8.4 ± 5.5 and females: 4.8 ± 6.3, $p = .282$). The redecomposition of HD-sEMG signals of participants for whom fewer than 10 MUs were identified resulted in identification of 4.5 ± 3.6 additional MUs in JC and 2.0 ± 1.5 in MS. Other descriptive statistics related to MU data are presented in Table 2. Regarding the performance of the isometric ramps, there was no evidence of significant differences in mean absolute error between control and JC (1.2 ± 1.0% MVC vs. .7 ± .2% MVC, $p = .082$) or between control and MS trials (.8 ± .3% MVC vs. 1.1 ± 1.6% MVC, $p = .482$).

3.3 | Paired motor unit technique

3.3.1 | Jaw clenching

There were no significant effects of intervention ($p = .339$, $\eta_p^2 = .01$), sex ($p = .752$) or an interaction

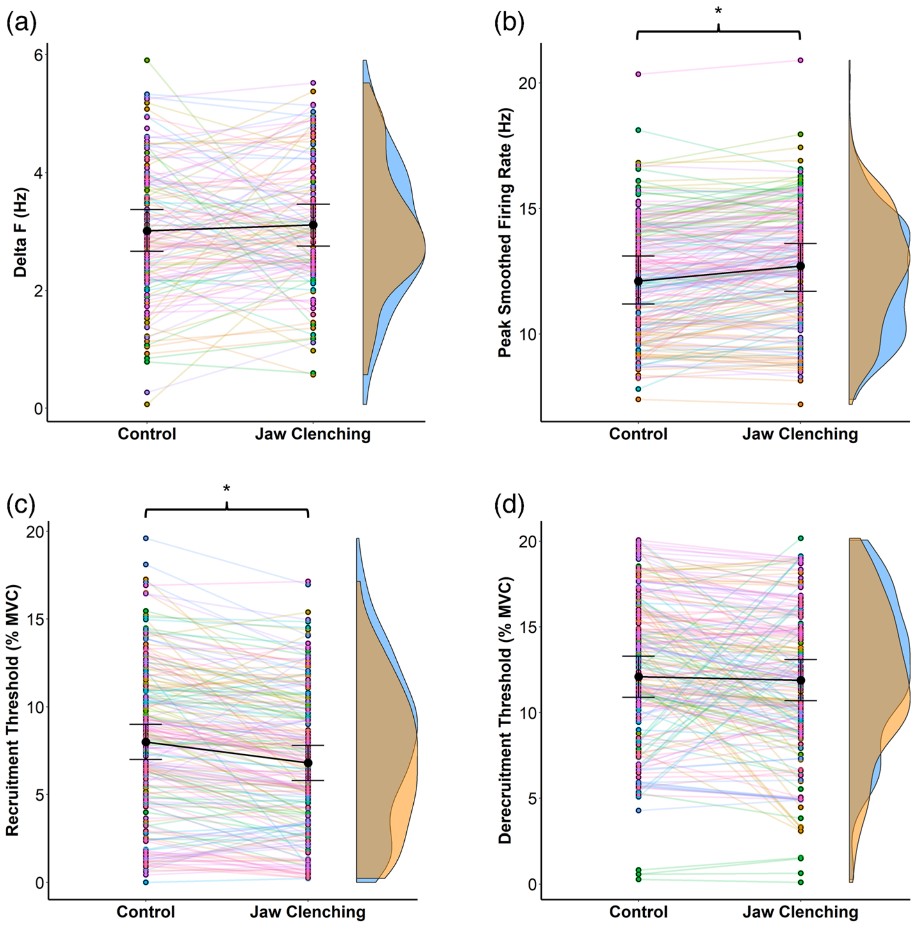

FIGURE 2 Data from the paired motor unit technique in control and jaw clenching trials. The different panels illustrate the changes in delta frequency (ΔF) of individual test units (a, $n = 18$ people, 130 test units), peak smoothed firing rate (b), and recruitment (c) and derecruitment thresholds (d) of individual motor units ($n = 18$ people, 212 motor units) from control to jaw clenching. Each pair of points represents an individual test unit (a) or motor unit (b–d), whilst each colour refers to one participant. Estimated marginal means are represented by black circles, with 95% confidence intervals indicated. Kernel density estimation (density curves) of the data is represented on the right by half-violin plots (blue for control and orange for jaw clenching). When considering all participants, the repeated measures nested linear mixed-effects models revealed a significant (*$p < .05$) increase in peak smoothed firing rate and a significant decrease in recruitment threshold from control to jaw clenching. Consideration of significant interactions revealed that the peak smoothed firing rate only increased in males, and the derecruitment threshold increased in females but decreased in males. MVC, maximal voluntary contraction.

between these two factors ($p = .643$) on ΔF (Figure 2a). The estimated marginal means of ΔF in control and JC were 3.01 Hz (2.66, 3.37) and 3.11 Hz (2.75, 3.46), respectively, with a mean difference of .10 Hz (−.10, .29). Females had an estimated marginal mean of ΔF at 3.11 Hz (2.58, 3.65), whilst males had a mean of 3.01 Hz (2.59, 3.42). The sex difference in ΔF was .10 Hz (−.78, .57). The recruitment threshold (torque at onset of MU firing) in control was not statistically associated with the change in ΔF (repeated measures correlation coefficient [r_{rm}] = −.02, $p = .831$). An effect of intervention was still not observed ($p = .401$) when 'sex' was not included as a fixed effect.

When considering all MUs in all participants, there was a 5.0% increase, $F(1, 210) = 34.22$, $p < .001$, $\eta_p^2 = .14$, in peak smoothed firing rate (Figure 2b) from control (12.1 Hz [11.2, 13.1]) to JC (12.7 Hz [11.7, 13.6]), with a mean difference of .51 Hz [.33, .68]. There was no main effect of sex ($p = .643$) or interaction between sex and intervention ($p = .943$). The recruitment threshold in control was not statistically associated with the change in peak smoothed firing rate ($r_{rm} = .07$, $p = .363$).

For all MUs, a significant, $F(1, 210) = 63.66$, $p < .001$, $\eta_p^2 = .23$, decrease in recruitment threshold (Figure 2c) from control (8.0% MVC [7.0, 9.0]) to JC (6.8% MVC [5.8, 7.8]) was detected, with a mean difference of −1.2% MVC [−1.6, −.9], but no significant effect of sex ($p = .166$) or sex × intervention interaction ($p = .280$). The decrease in recruitment threshold was more evident in MUs recruited later in the contraction, given the moderate

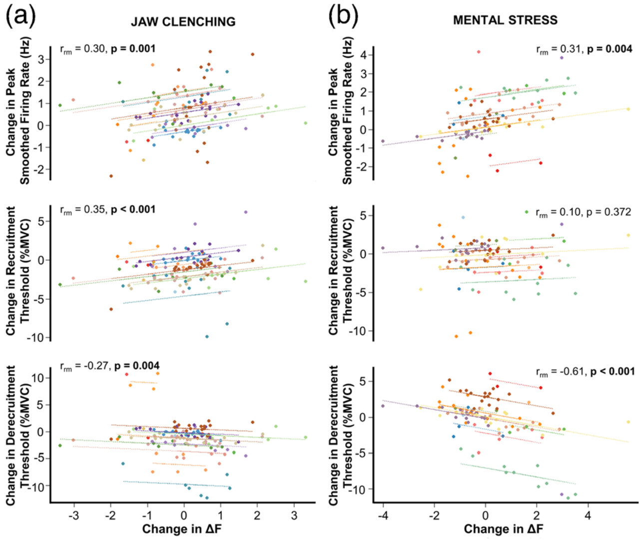

FIGURE 3 Repeated measures correlations plots illustrating the association between changes in delta frequency (ΔF) and changes in other dependent variables, in the jaw clenching (JC) condition (a, $n = 18$ people, 130 test units) and in the mental stress (MS) condition (b, $n = 14$ people, 100 test units). The non-independent nature of the observations is taken into consideration by the repeated measures correlation coefficient (r_{rm}), with separate parallel lines fitted to the data from each participant and represented by different colours. In both conditions, a greater increase in ΔF of test units was associated with a greater increase in their peak smoothed firing rate and a greater decrease in their derecruitment threshold. In JC, but not in MS, a greater increase in ΔF of test units was also associated with a greater increase in their recruitment threshold. MVC, maximal voluntary contraction.

association between the recruitment threshold in control and the change in recruitment threshold ($r_{rm} = -.42$, $p < .001$).

There was no effect of intervention (Figure 2d, $p = .330$, ${\eta_p}^2 < .01$) or sex ($p = .918$) on derecruitment threshold (torque at cessation of MU firing). Nonetheless, a significant interaction between sex and intervention was observed, $F(1, 210) = 49.93$, $p < .001$. In males, there was a significant decrease in derecruitment threshold (−1.9% MVC [−2.4, −1.4], $p < .001$) from control (12.9% MVC [11.3, 14.5]) to JC (11.0% MVC [9.4, 12.6]). On the contrary, a significant increase in derecruitment threshold (1.4% MVC [.7, 2.2], $p = .003$) was observed in females from control (11.3% MVC [9.6, 13.1]) to JC (12.8% MVC [11.0, 14.5]). However, this significant increase in females was driven by two out of eight participants with a large increase in derecruitment threshold in all MUs (note steep increasing lines in Figure 2d). There were no significant differences between males and females in control ($p = .187$) or JC ($p = .133$). The recruitment threshold in control was weakly associated with the change in derecruitment threshold ($r_{rm} = -.36$, $p < .001$).

An examination of whether changes in ΔF were accompanied by changes in other dependent variables (Figure 3a) revealed weak but significant correlations. These indicated that a greater increase in ΔF of test units was associated with a greater increase in their peak smoothed firing rate ($r_{rm} = .30$, $p = .001$), with a smaller decrease in their recruitment threshold ($r_{rm} = .35$, $p < .001$), with a greater decrease in their derecruitment threshold ($r_{rm} = -.27$, $p = .004$) and vice versa. There was no evidence that ΔF was statistically associated with the recruitment threshold of test units in control ($r_{rm} = -.03$, $p = .734$) or JC ($r_{rm} = .08$, $p = .428$).

3.3.2 | Mental stress

There was no effect of intervention ($p = .147$, ${\eta_p}^2 = .02$), or sex ($p = .700$), or intervention × sex interaction ($p = .351$) on ΔF (Figure 4a). The estimated marginal means of ΔF in control and MS were 3.22 Hz [2.80, 3.65] and 3.47 Hz [3.05, 3.90], respectively, with a mean difference of .25 Hz [−.09, .60]. Females had an estimated marginal mean of ΔF at 3.42 Hz [2.76, 4.08], whilst males had a mean of 3.28 Hz [2.86, 3.70]. The sex difference in ΔF was .14 Hz [−.92, .64]. The recruitment threshold in control was not statistically associated with the change in ΔF ($r_{rm} = .15$, $p = .170$). An effect of intervention was still not observed ($p = .257$) when 'sex' was not included as a fixed effect.

For all MUs, there was a 4.0% increase, $F(1, 184) = 20.56$, $p < .001$, ${\eta_p}^2 = .10$, in peak smoothed firing rate (Figure 4b) from control (12.5 Hz [11.2, 13.8]) to MS (13.0 Hz [11.8, 14.3]), with a mean difference of .54 Hz [.30, .77]. There was no effect of sex ($p = .214$) or sex × intervention ($p = .784$). The recruitment threshold in control was not statistically associated with the change in peak firing rate ($r_{rm} = .04$, $p = .579$).

Recruitment threshold decreased significantly, $F(1, 184) = 11.45$, $p < .001$, ${\eta_p}^2 = .06$ (Figure 4c), from control (7.7% MVC [6.5, 8.9]) to MS (7.0% MVC [5.8, 8.2]), with a mean difference of −.7% MVC [−1.1, −.3], but no significant effect of sex ($p = .690$) or sex × intervention interaction ($p = .586$) was observed. The decrease in recruitment threshold was more evident in MUs recruited later in the contraction, given the weak association between the recruitment threshold in control and the change in recruitment threshold ($r_{rm} = -.22$, $p = .005$).

There were no effects of intervention (Figure 4d, $p = .780$, ${\eta_p}^2 < .001$) or sex ($p = .291$) on derecruitment

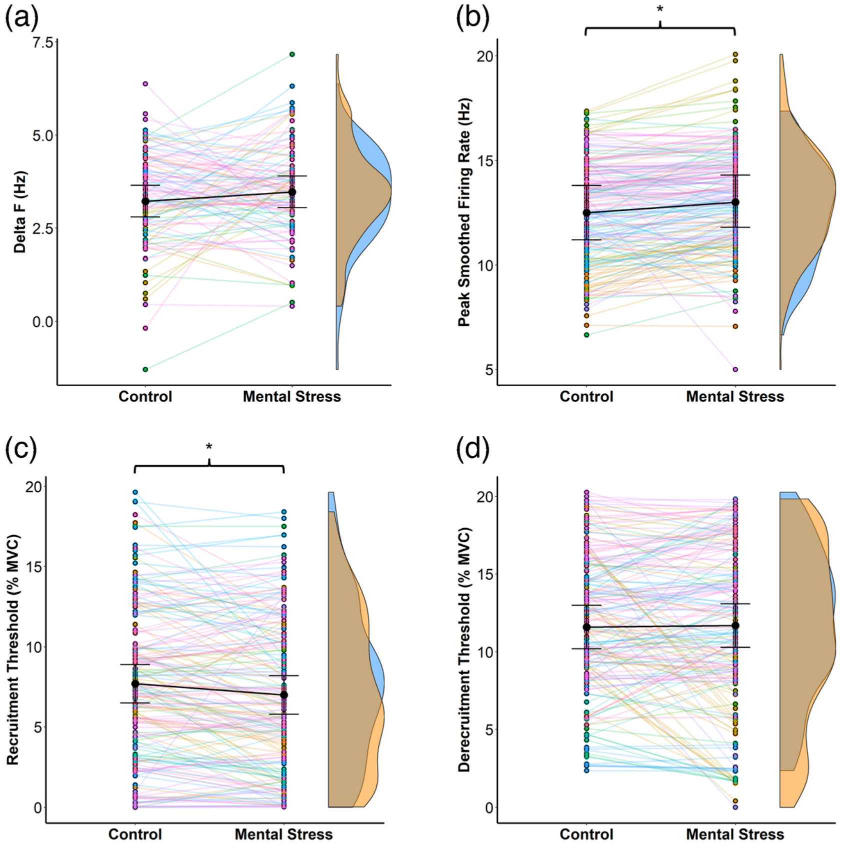

FIGURE 4 Data from the paired motor unit technique in control and mental stress trials. The different panels illustrate the changes in delta frequency (ΔF) of individual test units (a, $n = 14$ people, 100 test units), peak smoothed firing rate (b), recruitment (c) and derecruitment thresholds (d) of individual motor units ($n = 17$ people, 186 motor units) from control to mental stress. Each pair of points represents an individual test unit (a) or motor unit (b–d), whilst each colour refers to one participant. Estimated marginal means are represented in black circles, with 95% confidence intervals indicated. Kernel density estimation (density curves) of the data is represented on the right by half-violin plots (blue for control and orange for mental stress). When considering all participants, the repeated measures nested linear mixed-effects models revealed a significant (*$p < .05$) increase in peak smoothed firing rate and a decrease in recruitment threshold from control to mental stress. Consideration of significant interactions revealed that the derecruitment threshold was increased in females but decreased in males. MVC, maximal voluntary contraction.

threshold. Nonetheless, a significant interaction between sex and intervention was observed, $F(1, 184) = 31.00$, $p < .001$. In males (132 MUs), derecruitment threshold decreased (−1.5% MVC [−2.1, −.9], $p < .001$) from control (11.7% MVC [10.0, 13.4]) to MS (10.2% MVC [8.5, 11.9]). On the contrary, derecruitment threshold increased (+1.6% MVC [.7, 2.6], $p < .001$) in females (54 MUs) from control (11.5% MVC [9.3, 13.7]) to MS (13.2% MVC [11.0, 15.4]). There were no significant differences between males and females in control ($p = .899$), but derecruitment threshold was significantly higher in females than males in MS ($p = .039$). The recruitment threshold in control was not statistically associated with the change in derecruitment threshold ($r_{rm} = -.01$, $p = .879$).

An examination of whether changes in ΔF were accompanied by changes in other dependent variables (Figure 3b) revealed the following: (1) a weak but significant correlation ($r_{rm} = .31$, $p = .004$) indicating that a greater increase in ΔF of test units was associated with a greater increase in their peak smoothed firing rate and vice versa; (2) no significant correlation ($r_{rm} = .10$, $p = .0372$) between the change in ΔF of test units and the change in their recruitment threshold; and (3) a moderate correlation ($r_{rm} = -.61$, $p < .001$) indicating a greater increase in ΔF of test units associated with a greater decrease in their derecruitment threshold. There was no evidence that ΔF was statistically associated with the recruitment threshold of test units in control ($r_{rm} = .01$, $p = .924$) or MS ($r_{rm} = .13$, $p = .217$).

3.4 | VibStim

3.4.1 | Jaw clenching

One participant was excluded from analysis due to voluntary contractions of the leg during the trials, and the EMG-related variables from another participant were excluded due to technical problems (torque variables: $n = 20$; EMG variables: $n = 19$). A significant increase from control to JC was observed for all variables (Figure 5): T_{vib} (3.3% MVC [1.0, 5.7] vs. 10.4% MVC [5.7, 15.2], $p = .001$), EMG_{vib} (22.65 μV [15.41, 29.88] vs. 37.05 μV [25.02, 49.08], $p < .001$), $T_{sust0.5}$ (1.8% MVC [.0, 3.5] vs. 7.5% MVC [3.3, 11.6], $p = .002$), $EMG_{sust0.5}$ (16.89 μV [12.63, 21.16] vs. 28.21 μV [19.62, 36.80], $p = .001$), T_{sust3} (1.2% MVC [.0, 2.5] vs. 6.0% MVC [2.4, 9.5], $p = .002$), EMG_{sust3} (15.31 μV [11.59, 19.05] vs. 23.81 μV [16.10, 31.52], $p = .011$), and warm-up (1.2% MVC [.1, 2.3] vs. 4.2% MVC [2.6, 5.6], $p < .001$). No significant effects of sex ($p = .054$–.671) or a sex × intervention interaction

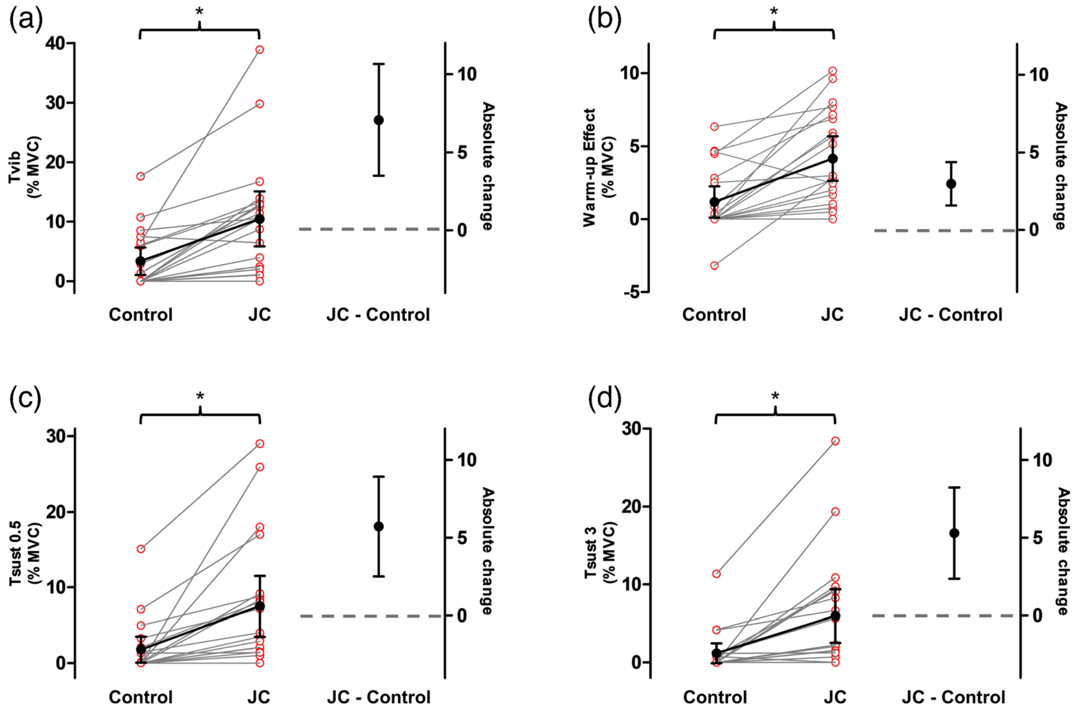

FIGURE 5 Changes in the torque-related variables from the control to the jaw clenching (JC) trials during tendon vibration with superimposed neuromuscular electrical stimulation (VibStim). All torques are expressed relative to maximal voluntary contraction (MVC) torque. On the left side of the graphs, red open circles represent data from individuals with grey lines joining their control and JC values ($n = 20$). Means of control and JC trials are shown by black filled circles with 95% confidence intervals. Mean change is shown by a black line. The right side of the graphs shows the mean difference between JC and control (black full circle) with 95% confidence intervals. Data on the left side of the graphs are plotted against the left y axis and data on the right against the right y axis, with a grey horizontal dashed line indicating $y = 0$ for the right y axis. T_{vib} (a), reflexive torque during vibration; warm-up (b), difference between T_{vib} and torque after the first burst of neuromuscular electrical stimulation; $T_{sust0.5}$ (c) and T_{sust3} (d), self-sustained torque 0.5 and 3 s after vibration, respectively. There was evidence of a significant ($*p < .05$) increase in all variables from control to JC.

($p = .086$–$.940$) were observed for any variable. Excluding participants who did not exhibit an involuntary evoked torque in the time window of interest during control did not change the outcomes.

3.4.2 | Mental stress

One participant was excluded from analysis due to voluntary contractions of the leg during the trials, and another participant withdrew from the study before the completion of this condition ($n = 19$). Significant effects of MS on T_{vib}, $F(1, 17) = 8.42$, $p = .010$, $\eta_p^2 = .331$; EMG_{vib}, $F(1, 17) = 10.43$, $p = .005$, $\eta_p^2 = .380$; $T_{sust0.5}$, $F(1, 17) = 6.98$, $p = .017$, $\eta_p^2 = .291$; and warm-up, $F(1, 17) = 10.147$, $p = .005$, $\eta_p^2 = .374$, were observed (Figure 6). These variables increased from control to MS (T_{vib}: 4.5% MVC [1.5, 7.5] vs. 7.1% MVC [3.1, 11.2], EMG_{vib}: 21.35 μV [11.43, 31.26] vs. 28.59 μV [17.95, 39.22], $T_{sust0.5}$: 2.8% MVC [0, 5.6] vs. 4.5% MVC [1.0, 8.0], and warm-up: .8% MVC [−.2, 1.9] vs. 3.1% MVC [1.2, 4.9]). No significant effects of the intervention were observed for other variables: $EMG_{sust0.5}$ (20.32 μV [11.32, 29.32] vs. 22.27 μV [14.03, 30.51], $p = .201$), T_{sust3} (2.1% MVC [.0, 4.7] vs. 3.3% MVC [.0, 6.7], $p = .103$) and EMG_{sust3} (17.20 μV [9.89, 24.51] vs. 19.39 μV [11.71, 27.06], $p = .119$). A significant interaction between sex and intervention, $F(1, 17) = 5.90$, $p = .027$, $\eta_p^2 = .258$, was observed for EMG_{sust3}, revealing a significant increase from control to MS in males (22.29 μV [11.68, 32.90] vs. 27.71 μV [16.58, 38.84], $p = .012$), but not in females (12.11 μV [2.04, 22.17] vs. 11.06 μV [.50, 21.6], $p = .576$). EMG_{sust3} was not different between males and females at control ($p = .160$) but was higher in males in MS ($p = .035$). There was no significant sex × intervention interaction ($p = .180$–$.618$) for any other variable nor an effect of sex ($p = .072$–$.324$) for any variable. In an exploratory analysis with exclusion of participants with $T_{sust0.5} < 1\%$ MVC during control, the increase in $T_{sust0.5}$ from control to MS became non-significant ($5.3 \pm 7.8\%$ MVC vs. $7.6 \pm 9.6\%$ MVC, $p = .094$, $n = 9$). The outcomes in the other variables did not change.

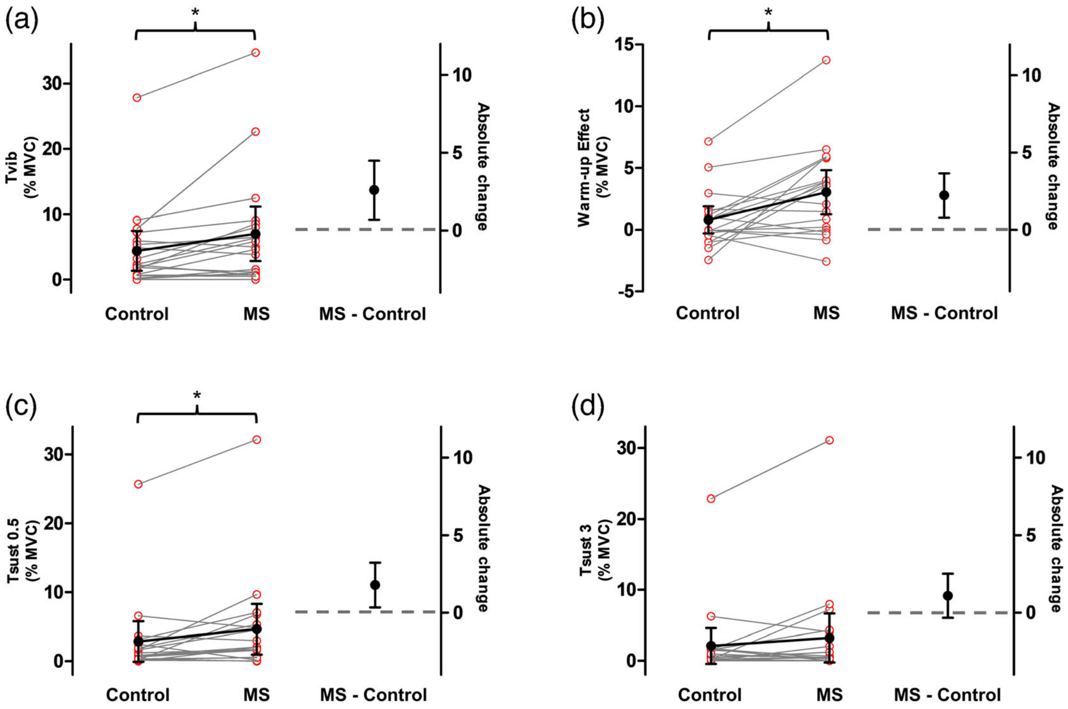

FIGURE 6 Changes in the torque-related variables from the control to the mental stress (MS) trials during tendon vibration with superimposed neuromuscular electrical stimulation (VibStim). On the left side of the graphs, red open circles represent data from individuals ($n = 19$), normalised to maximal voluntary contraction (MVC) torque, and grey lines represent individual changes. Means of control and MS trials are shown in black filled circles, with 95% confidence intervals indicated. Mean change is shown by a black line. On the right side of the graphs, the mean difference between MS and control is shown in a black full circle, with 95% confidence intervals of the mean difference indicated. Data on the left side of the graphs are plotted against the left-hand y axis, and data on the right side of the graphs are plotted against the right-hand y axis, with a grey horizontal dashed line indicating $y = 0$ for the right y axis. T_{vib} (a), reflexive torque during vibration; warm-up (b), difference between T_{vib} and torque after the first burst of neuromuscular electrical stimulation; $T_{sust0.5}$ (c) and T_{sust3} (d), self-sustained torque 0.5 and 3 s after vibration, respectively. There was evidence of a significant (*$p < .05$) increase in T_{vib} (a), warm-up (b) and in $T_{sust0.5}$ (c) from control to MS, but not in T_{sust3} (d).

3.5 | Manipulation check and heart rate

There was a significant increase in self-rated stress in JC (Figure 7a) during both VibStim, $F(1, 18) = 23.86$, $p < .001$, $\eta_p^2 = .570$, and paired MU trials, $F(1, 16) = 26.11$, $p < .001$, $\eta_p^2 = .620$. In VibStim, self-rated increased from 3.5 [2.9, 4.0] to 4.8 [4.2, 5.4], with no effect of sex ($p = .547$) or sex × intervention interaction ($p = .712$). In the paired MU technique, self-rated stress increased from 2.4 [1.9, 2.8] to 3.8 [3.1, 4.4], and there was no effect of sex ($p = .360$) or an interaction effect ($p = .238$).

In MS, there was also a significant increase in self-rated stress (Figure 7b) during both VibStim, $F(1, 17) = 56.24$, $p < .001$, $\eta_p^2 = .852$, and paired MU trials, $F(1, 16) = 86.68$, $p < .001$, $\eta_p^2 = .620$. In VibStim, self-rated stress increased from 3.4 [2.9, 3.9] to 5.4 ([5.0, 5.8], with no effect of sex ($p = .517$) or sex × intervention interaction ($p = .957$). In the paired MU technique, self-rated stress increased from 2.7 [2.9, 3.2]) to 5.2 ([4.7, 5.6), with no effect of sex ($p = .360$) or a sex × intervention interaction ($p = .238$).

A significant increase in heart rate in JC, $F(1, 17) = 38.61$, $p < .001$, $\eta_p^2 = .694$, was observed, with no effect of sex ($p = .292$) or sex × intervention interaction ($p = .910$). Heart rate increased from 74 bpm [67, 81] to 82 bpm [75, 88]. In MS, both a significant intervention effect, $F(1, 17) = 41.09$, $p < .001$, $\eta_p^2 = .733$, and a sex × intervention interaction, $F(1, 17) = 6.035$, $p = .027$, $\eta_p^2 = .287$, were observed. Heart rate increased in both males and females, but this increase was larger ($p = .027$) in males (72 bpm [63, 83] to 92 bpm [81, 103], $p < .001$) than in females (79 bpm [71, 88] to 88 bpm [78, 97], $p = .008$). In JC, there were no significant correlations between the changes in absolute or relative (% of predicted maximum heart rate; 220 bpm – age) heart rate and changes in the dependent variables when using VibStim. However, in MS, significant positive correlations

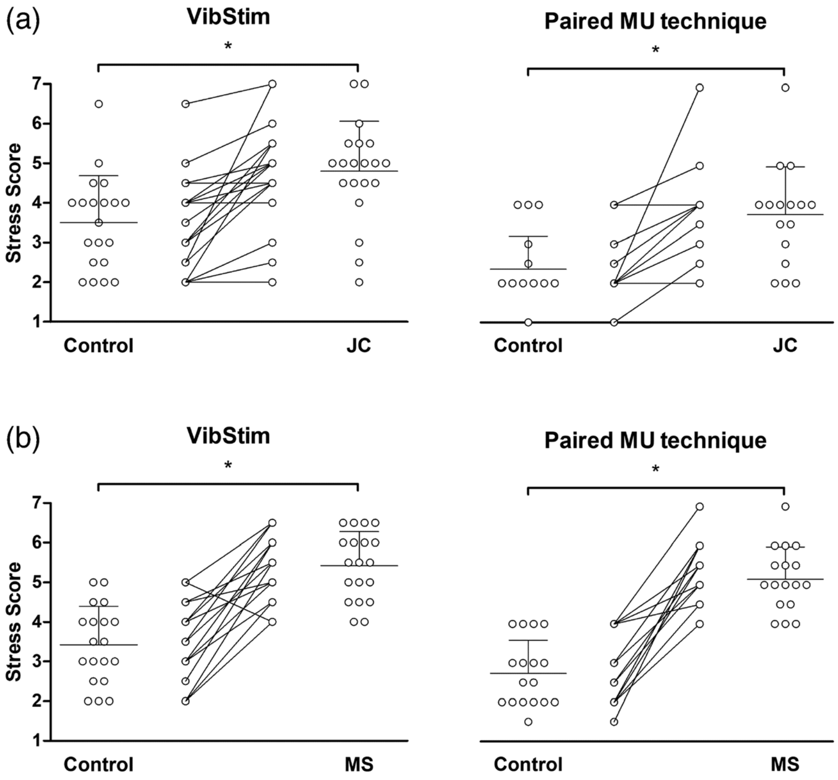

FIGURE 7 Individual stress scores reported during control and experimental trials for jaw clenching (JC) (a, $n = 19$) and mental stress (MS) (b, $n = 19$) conditions. Participants were asked to indicate how relaxed or stressed they felt during each trial. Stress scores were significantly ($^*p < .05$) higher in JC and MS trials. Symbols represent data from individuals. On the left- and right-hand side of each graph, the number of circles corresponds to the number of participants that indicated each score. On the centre of each graph, within-participant changes are depicted. Means are shown in horizontal lines with standard deviations indicated. MU, motor unit; VibStim, tendon vibration with superimposed neuromuscular electrical stimulation.

were found between the changes in heart rate and changes in both $T_{sust0.5}$ ($r = .605$, $p = .010$) and T_{sust3} ($r = .559$, $p = .020$).

4 | DISCUSSION

We hypothesised that serotonin and noradrenaline release induced by jaw clenching and mental stress would enhance the contribution of PICs to motoneuron firing. We found that these interventions increased both plantar flexor torque and muscle activity measured during involuntary contractions (VibStim) but did not influence firing rate hysteresis measured during voluntary, ramp contractions in GM MUs (paired MU technique). Thus, effects of JC and MS were observed only in VibStim. This lack of consistency between the techniques might theoretically be explained by the following: (1) different organisation and magnitudes of ionotropic and neuromodulatory inputs to motoneurons in a voluntary task versus evoked contractions, suggesting that the effect of monoaminergic interventions on PIC activity might depend on the background level of neuromodulation as well as excitatory and inhibitory inputs to the motoneurons; (2) differential effects of the interventions on plantar flexor synergists; (3) the interventions not sufficiently increasing monoamine release onto motoneurons; (4) mechanism(s) other than increased PICs in alpha motoneurons contributing to the effects in VibStim; or (5) a higher sensitivity of VibStim than the paired MU technique to reflect PIC activation during maximal jaw clenching and mental stress.

4.1 | Do jaw clenching and mental stress increase neuromodulator release on motoneurons?

Spinal motoneurons receive diffuse serotonergic innervation from the raphe nuclei (Alvarez et al., 1998; Bowker et al., 1981), and serotonin release is likely increased during motor activity (Jacobs et al., 2002; Veasey et al., 1995). Thus, contraction of one muscle could increase the gain at the motoneuron pool of another muscle. In the present study, it was not possible to verify that additional serotonin was released by the remote contraction due to the absence of a suitable biomarker (Kavanagh & Taylor, 2022). Nonetheless, we provide indirect evidence that jaw clenching leads to an increase in neuromodulation through greater involuntary torque and muscle activity magnitudes in VibStim and earlier recruitment and greater firing rates of MUs during ramp contractions.

Motoneurons also receive neuromodulatory input from the LC (Proudfit & Clark, 1991), and the activity of LC noradrenergic neurons correlates with arousal state

(Rajkowski et al., 1997), increasing in response to stress (Valentino & Van Bockstaele, 2008). LC activation results in complex patterns of far-reaching neuronal activity (Poe et al., 2020). Accordingly, LC activity can be estimated in humans through behavioural responses such as pupil dilation (Joshi et al., 2016) or heart rate increases (Gurtu et al., 1984; Ter Horst et al., 1991). Furthermore, it is expected that changes in these autonomic responses would be reflected in changes in noradrenaline release onto spinal motoneurons. In the present study, the increased levels of self-rated stress, higher heart rates, and clear motor effects provide indirect evidence that MS led to increased LC activity with a consequent neuromodulatory effect on the motoneurons. MS had positive effects on most VibStim variables and modulated certain MU parameters during ramp contractions, indicating higher motoneuron excitability. Interestingly, self-sustained muscle activity measured 3 s after vibration cessation was increased in males but not in females. This might have resulted from greater stress induced by the countback task in males than females, as indicated by greater heart rate responses in males. This observation along with the positive correlations found between MS-induced changes in heart rate and changes in both $T_{sust0.5}$ and T_{sust3} suggest that the observed increases in self-sustained activity in VibStim resulted from enhanced neuromodulation. The unexpected increase in stress levels during JC is suggestive that both serotonin and noradrenaline might have been released onto spinal motoneurons to a greater extent in this intervention, resulting in a cumulative neuromodulatory effect and partly explaining the more pronounced effect of JC than MS in VibStim.

The increases in VibStim variables are consistent with our hypothesis. It should be noted that an increase in monoaminergic release during JC or MS remains speculative and other mechanisms might have partly contributed to these effects (discussed below). Nonetheless, an increase in PICs best explains the observed effects. VibStim has been proposed as an alternative to the paired MU technique to estimate the contribution of PICs to motoneuron firing in humans (Trajano et al., 2014). As PIC channels are voltage-gated, their opening should be triggered by excitatory Ia afferent input in humans (Gorassini et al., 1998). The ability to estimate PICs in humans both during voluntary and involuntary contractions would hypothetically make the VibStim and paired MU techniques complementary. However, our findings did not reveal parallel changes in the PIC estimates provided by these techniques because the interventions did not influence ΔF measured during the voluntary, ramp contractions.

4.2 | Lack of change in ΔF

Greater levels of neuromodulatory inputs to the spinal cord lead to larger PICs with greater effects on motoneuron firing (Conway et al., 1988; Hounsgaard et al., 1988). However, contrary to our hypothesis, no significant changes in ΔF during voluntary, ramp contractions were detected in JC or MS. To the best of our knowledge, this is the first study to investigate the effects of jaw clenching on ΔF. Regarding MS, no effect on ΔF is consistent with the lack of effect of acute psychosocial stress on ΔF in trapezius MUs acquired with bipolar intramuscular recordings (Stephenson & Maluf, 2010).

Given that the current interventions increased both torque and EMG variables in the absence of voluntary drive (VibStim), it is possible that a neuromodulatory ceiling was reached during the submaximal voluntary contractions, limiting further PIC amplification. Greater serotonergic (Jacobs et al., 2002; Veasey et al., 1995) and noradrenergic (Noga et al., 2017) input to motoneurons would be expected during the voluntary ramp contractions (paired MU technique) than during the involuntary VibStim contractions. Speculatively, further neuromodulation of GM motoneurons might have been less effective during the interventions due to saturation of brainstem output, as previously observed in walking cats (Veasey et al., 1995), or monoamine receptors. This would be consistent with a recent study, in which a ceiling effect of neuromodulation of human GM motoneurons was proposed after observing an increase in ΔF from 10% to 20% MVC ramps but not from 20% to 30% (Orssatto, Mackay, et al., 2021).

The discrepancy in results between methods might also be explained by a differential effect on triceps surae components. HD-sEMG signals were decomposed only from GM, and in fact, there was little GM EMG at the end of the ramp contractions in some participants. Although speculative, the minimal common drive between soleus, GM and gastrocnemius lateralis (Hug et al., 2021) could have led to muscle-specific increases in synergist PICs rather than GM PICs. A previous study found that soleus but not GM ΔF values were reduced after brief plantar flexor stretches (Trajano et al., 2020).

Differential modulation of the contribution of PIC activity to motoneuron firing might have been expected across the motoneuron pool (Mesquita et al., 2020). However, changes in ΔF were not associated with MU recruitment threshold. This lack of association was also observed during reciprocal inhibition and whole-body relaxation (Mesquita et al., 2022), but an association was evident in tibialis anterior MUs during local vibration (Lapole et al., 2023). Future studies could examine the effects of these interventions in motoneuron pools with different proportions of smaller motoneurons and across a wide range of contraction intensities. Moreover, it remains unclear whether there are sex differences in PICs. The absence of differences in GM ΔF between males and females reported in our current and previous studies (Mesquita et al., 2022) contrasts with recent evidence of greater ΔF in lower limb muscles of females (Jenz et al., 2023). However, current observations are based on small sample sizes (up to 9 vs. 9 in our observations and 10 vs. 10 in Jenz et al., 2023) and future studies could usefully include a greater number of females and males. Another important factor to consider in the future is the possible effect of sex hormone variation during the menstrual cycle on synaptic inhibition (Haage et al., 2002), neuromodulation (Bethea et al., 2002; Koldzic-Zivanovic et al., 2004; Smith et al., 2004) and, thus, PIC activity.

PIC channels are activated below the motoneuron recruitment threshold (Afsharipour et al., 2020; Bennett et al., 1998; Bennett, Li, & Siu, 2001), and subthreshold PIC modulation may affect motoneuron behaviour. A decrease in MU recruitment threshold was observed in both JC and MS. This change is consistent with an increase in subthreshold PICs, which might be expected to increase the number of motoneurons near spiking threshold at any given level of central drive (Škarabot et al., 2019), allowing motoneurons to be recruited at lower levels of net input. This could theoretically be induced by greater neuromodulation. In addition, a greater level of partial PIC activation before motoneuron recruitment may decrease recruitment–derecruitment hysteresis and hence reduce the computed ΔF score, which estimates the contribution of *suprathreshold* PICs to motoneuron firing. This has not been proven, although some support is provided by the finding that MUs in JC showing the greatest recruitment threshold decrease also showed the greatest ΔF decrease (Figure 3a, middle graph). It can then be postulated that during rapid, maximal contractions, a greater motoneuron recruitment speed (i.e., a more compressed recruitment range) induced by greater subthreshold PIC activation explains the positive effects of JC on the rate of force development (Del Vecchio et al., 2021; Dideriksen et al., 2020), in exercises such as countermovement jump (Ebben et al., 2008) and back row exercise (Buscà et al., 2016). Alternatively, the simultaneous maximal, sustained jaw clenching and voluntary plantarflexion contraction may have resulted in serotonin release of sufficient magnitude to spill over from the somato-dendritic compartment onto inhibitory 5-HT$_{1a}$ receptors at the axon initial segment of the motoneurons (Cotel et al., 2013), attenuating the otherwise amplifying effects of PICs on motoneuron output. Accordingly, a recent study in humans has suggested greater activation of 5-HT$_{1a}$ receptors during maximal but not submaximal fatiguing contractions (Henderson et al., 2022). Hence, performing JC or another remote voluntary contraction at a submaximal level (e.g., 40% MVC, as per Orssatto et al., 2022, and Mackay-Phillips et al., 2022) may have resulted in ΔF increases. However, it is not possible to directly quantify the potential effect of 5-HT$_{1a}$ receptor activation in the current study.

PICs are exquisitely sensitive to inhibition (Bui et al., 2008; Hounsgaard et al., 1988; Hultborn et al., 2003; Hyngstrom et al., 2007; Kuo et al., 2003; Mesquita et al., 2022; Orssatto et al., 2022; Revill & Fuglevand, 2017; Vandenberk & Kalmar, 2014). Thus, we cannot rule out the possibility that the lack of a significant ΔF change resulted from a concomitant increase in inhibition during the ramp contractions. Whilst increased ΔF scores have been observed in pharmacological neuromodulation studies (D'Amico et al., 2013; Udina et al., 2010), the requirement to accurately follow the force trace may have engaged a compensatory inhibition to attenuate the effects of increased neuromodulation (Heckman, Hyngstrom, & Johnson, 2008). Consequently, detection of recruitment–derecruitment hysteresis would be less likely.

Despite the absence of consistent ΔF changes, ΔF for individual MUs changed variably from control to experimental conditions (see Figures 2a and 4a). The underlying reasons for this variability are unknown. It is possible that different motoneurons were differentially influenced by the previously mentioned mechanisms of neuromodulatory ceiling, subthreshold PIC activation, serotonin spill-over and compensatory inhibition. Nonetheless, increases in peak smoothed firing rates correlated positively with changes in ΔF in both conditions, suggesting that MUs exhibiting greater recruitment–derecruitment hysteresis also tended to fire faster. Thus, changes in these two discharge properties could have been induced by the same underlying increase in PIC activity. As expected, decreases in derecruitment threshold were associated with greater increases in ΔF in both conditions, suggesting that MUs derecruited at a lower level of synaptic input were also derecruited at a lower level of force. This observation supports the functional relevance of PIC-induced hysteresis, which allows prolonged motoneuron firing at lower levels of force and is likely important to facilitate steady motor output during posture and stabilisation tasks.

Finally, several limitations of the current methodology should be considered when interpreting these and other MU parameters. First, changes in MU parameters are interdependent and their interpretation is complex, as participants were asked to follow a force trace. The firing behaviour of a given MU will depend on the firing frequency and number of previously recruited MUs, and any changes in MU behaviour may elicit compensatory changes in the voluntary drive so that the task is performed accurately. Second, the MUs identified through HD-sEMG signal decomposition represent a relatively small portion of the total number of GM motoneurons actively contributing to the contraction, with a bias towards MUs with the largest surface action potentials (Farina et al., 2010). Consequently, other non-identified MUs may not have behaved similarly. Third, synergistic muscles also contributed to ramp force generation (Hug et al., 2021). These synergists receive minimal common drive, and it is possible that they were differentially affected by the interventions (Hali et al., 2021). Fourth, EMG from antagonistic muscles was not collected, and an increase in co-contraction may have explained a concomitant increase in peak smoothed firing rate and lower recruitment thresholds in both interventions. Finally, known limitations of the paired MU technique should be taken into consideration when interpreting our findings. The quantified firing rate hysteresis can be contaminated by spike frequency adaptation (Powers & Heckman, 2015; Revill & Fuglevand, 2011; Vandenberk & Kalmar, 2014) and by changes in the firing rate profile of the control units and is generally confined to relatively higher threshold MUs (Beauchamp et al., 2023).

4.3 | PICs and alternative underlying mechanisms for the observed effects on motor output

The hypothesis that greater motoneuron PIC activity explains the increase in VibStim variables is consistent with findings from intracellular recordings in animals (Lee & Heckman, 2000) showing dendritic amplification of Ia afferent inputs with moderate and strong neuromodulatory drive. Alternatively, greater monoamine release onto motoneurons could have decreased the afterhyperpolarisation amplitude (Berger et al., 1992), generated a slow subthreshold depolarisation (Wang & Dun, 1990) or shifted the motoneuron voltage threshold towards a more hyperpolarised membrane potential (Krawitz et al., 2001). Collectively, these might increase motoneuron excitability and thus both muscle torque and activity. Moreover, increases in monoaminergic drive may influence afferent input from the muscle. For example, tendon tap reflexes in plantar flexor muscles increase during a stressful countback task similar to the one used in the current study (Hjortskov et al., 2005). This might be explained by increased activity of noradrenergic neurons acting directly on muscle spindles (Barker & Saito, 1981) or fusimotor outflow (i.e., increased activity of gamma motoneurons) leading to increased spindle sensitivity to muscle lengthening. Interestingly, both fusimotor drive in relaxed muscles (Ribot et al., 1986) and muscle spindle sensitivity during passive stretching (Ribot-Ciscar et al., 2000) increased during both the Jendrassik manoeuvre and mental computation. However, the authors concluded that these interventions would only affect the firing of gamma motoneurons sufficiently to be detectable at the level of muscle spindle output if the participants were completely relaxed in the control trials (Ribot-Ciscar et al., 2000). Thus, it would be unlikely that a change in fusimotor drive would have significantly contributed to the observed motor effects in the current study, given that the participants were not completely relaxed during the control trials. In any case, results from attempts to independently modulate fusimotor drive have been conflicting across studies in humans and this topic is still a matter of debate (Burke, 2021; Dimitriou, 2021).

Importantly, these alternative mechanisms cannot explain increases in self-sustained activity in VibStim (i.e., $T_{sust0.5}$, $EMG_{sust0.5}$, T_{sust3}, and EMG_{sust3}). One could argue that a modulation of resting firing rates of muscle spindles could explain an increase in self-sustained activity. However, this phenomenon of intrafusal stiction is usually observed after voluntary isometric contractions (Gregory et al., 1990; Wilson et al., 1995) and would be unlikely in the reflexive contractions in VibStim. On the contrary, muscle spindles tend to show a decrease in spontaneous firing rate immediately after vibration cessation (Ribot-Ciscar et al., 1998). Rather, increases in self-sustained activity might be explained by greater PIC-induced bistable behaviour in some motoneurons (Lee & Heckman, 1999). Nonetheless, self-sustained firing of spinal cord interneurons (Méndez-Fernández et al., 2020) possibly due to reverberating spinal circuits (Bellardita et al., 2017) cannot be ruled out. Moreover, it is also possible that only very low-threshold motoneurons increased PIC activity, as the PIC contribution to motoneuron firing cannot be estimated in these motoneurons in the paired MU technique (Beauchamp et al., 2023). To further clarify the sensitivity of VibStim to estimate PICs, future studies could attempt to decompose EMG signals during VibStim and track the identified MUs during a voluntary ramp contraction. Given that self-sustained firing and hysteretic firing phenomena are likely explicable by the same biophysical mechanisms (Binder et al., 2020), MUs with a greater ΔF (estimated during a voluntary ramp contraction) might be expected to also exhibit stronger self-sustained firing in the seconds after VibStim.

5 | CONCLUSION

The net contribution of suprathreshold PICs to motoneuron firing was not changed by jaw clenching or mental stress when assessed by changes in human GM MU firing characteristics during a voluntary, submaximal, high-accuracy motor task. During involuntary evoked contractions in the absence of voluntary drive to the muscles, however, PIC activation was likely increased by both jaw clenching and mental stress. Our findings suggest that neuromodulatory interventions might affect the strength of PIC activity differently during voluntary versus reflexive contractions or that their effects specifically on GM MUs are minimal. During a voluntary, ramp contraction, the increased neuromodulatory input associated with the voluntary drive and the compensatory inhibitory inputs needed to accurately follow the force trace trajectory might attenuate further PIC-induced amplification of synaptic inputs during neuromodulatory interventions such as jaw clenching and mental stress. The effects of enhancing monoaminergic inputs might thus depend on the background level of neuromodulation, as well as the sum of excitatory and inhibitory drive to the motoneurons.

AUTHOR CONTRIBUTIONS

Ricardo N. O. Mesquita: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; visualization; writing—original draft; writing—review and editing. **Janet L. Taylor:** Conceptualization; supervision; writing—review and editing. **Gabriel S. Trajano:** Conceptualization; supervision; writing—review and editing. **Aleš Holobar:** Resources; software; supervision; writing—review and editing. **Basílio A. M. Gonçalves:** Resources; software; writing—review and editing. **Anthony J. Blazevich:** Conceptualization; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

There are no competing interests, financial or otherwise, to report regarding this manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Non-identifiable datasets generated and/or analysed during the current study and scripts for semi-automated analysis of the data are available from the corresponding author on request.

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