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T. Lapole, R. Mesquita, S. Baudry, Robin Souron, C. Brownstein, et al.. Can local vibration alter the contribution of persistent inward currents to human motoneuron firing?. *The Journal of Physiology*, 2023, 601 (8), pp.1467-1482. 10.1113/jp284210 . hal-04080242v1

HAL Id: hal-04080242

<https://nantes-universite.hal.science/hal-04080242v1>

Submitted on 24 Apr 2023 (v1), last revised 30 Aug 2023 (v2)

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Can local vibration alter the contribution of persistent inward currents to human motoneuron firing?

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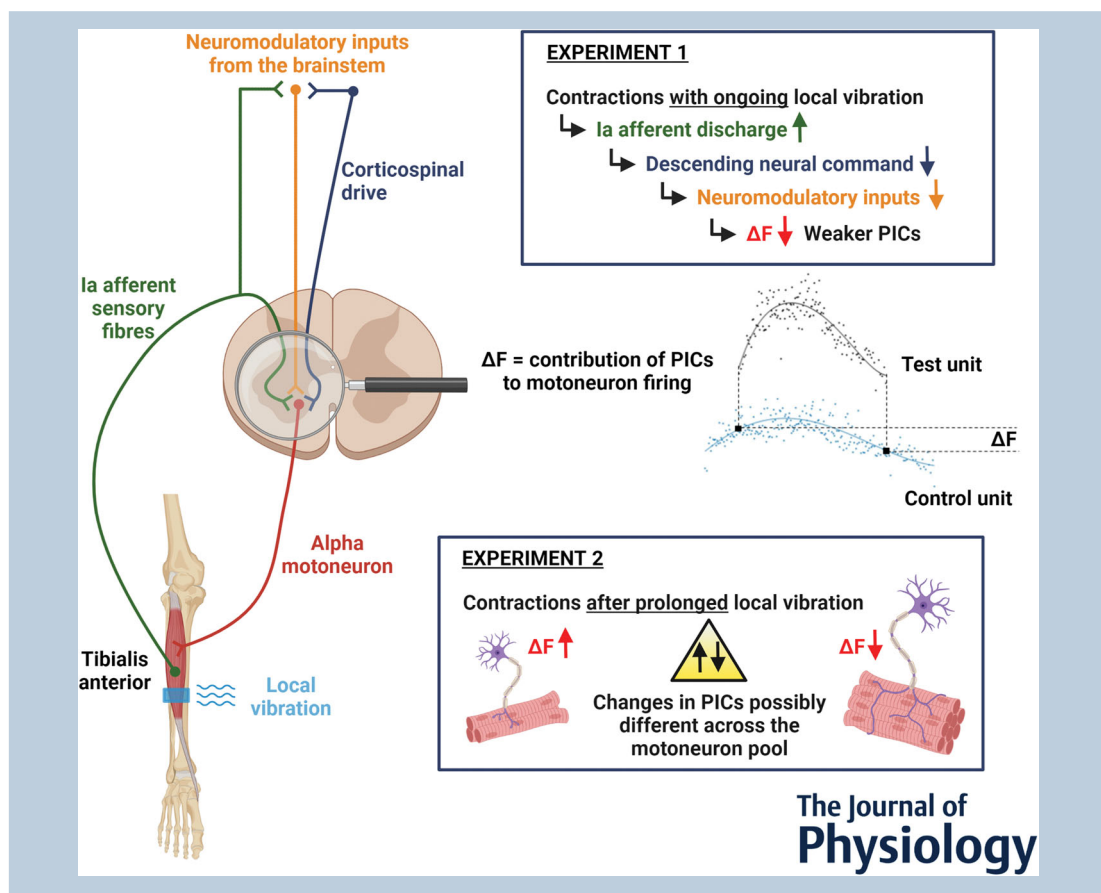
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Handling Editors: Richard Carson & Vaughan Macefield

The peer review history is available in the Supporting Information section of this article (<https://doi.org/10.1113/JP284210#support-information-section>).



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Abstract The response of spinal motoneurons to synaptic input greatly depends on the activation of persistent inward currents (PICs), which in turn are enhanced by the neuromodulators serotonin and noradrenaline. Local vibration (LV) induces excitatory Ia input onto motoneurons and may alter neuromodulatory inputs. Therefore, we investigated whether LV influences the contribution of PICs to motoneuron firing. This was assessed in voluntary contractions with concurrent, ongoing LV, as well as after a bout of prolonged LV. High-density surface electromyograms (HD-EMG) of the tibialis anterior were recorded with a 64-electrode matrix. Twenty males performed isometric, triangular, dorsiflexion contractions to 20% and 50% of maximal torque at baseline, during LV of the tibialis anterior muscle, and after 30-min of LV. HD-EMG signals were decomposed, and motor units tracked across time points to estimate PICs through a paired motor unit analysis, which quantifies motor unit recruitment–derecruitment hysteresis (ΔF). During ongoing LV, ΔF was lower for both 20% and 50% ramps. Although significant changes in ΔF were not observed after prolonged LV, a differential effect across the motoneuron pool was observed. This study demonstrates that PICs can be non-pharmacologically modulated by LV. Given that LV leads to reflexive motor unit activation, it is postulated that lower PIC contribution to motoneuron firing during ongoing LV results from decreased neuromodulatory inputs associated with lower descending corticospinal drive. A differential effect in motoneurons of different recruitment thresholds after prolonged LV is provocative, challenging the interpretation of previous observations and motivating future investigations.

(Received 8 December 2022; accepted after revision 23 February 2023; first published online 27 February 2023)

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Abstract figure legend Neuromodulatory inputs from the brainstem influence motoneuron intrinsic excitability through activation of persistent inward currents (PICs), making motoneurons more responsive to synaptic input. The ΔF method was used to estimate the contribution of PICs to the prolongation of human motoneuron firing. In experiment 1, we observed a lower contribution of PICs to motoneuron firing when vibration was concurrently applied during voluntary ramp dorsiflexions (participants were asked to follow a force trace). An increased contribution of vibration-induced reflex activation to force output (i.e. greater Ia afferent discharge) likely led to lower magnitudes of descending voluntary drive and neuromodulatory inputs. In experiment 2, prolonged exposure to vibration had different effects on the contribution of PICs to lower- vs. higher-threshold motor units in subsequent ramp contractions. Our results suggest that prolonged vibration can increase contribution of PICs to motoneuron firing in lower-threshold motor units, but decrease this contribution in higher-threshold motor units.

Key points

- Neuromodulatory inputs from the brainstem influence motoneuron intrinsic excitability through activation of persistent inward currents (PICs). PICs make motoneurons more responsive to excitatory input.
- We demonstrate that vibration applied on the muscle modulates the contribution of PICs to motoneuron firing, as observed through analysis of the firing of single motor units.
- The effects of PICs on motoneuron firing were lower when vibration was concurrently applied during voluntary ramp contractions, likely due to lower levels of neuromodulation. Additionally, prolonged exposure to vibration led to differential effects of lower- vs. higher-threshold motor units on PICs, with lower-threshold motor units tending to present an increased and higher-threshold motor units a decreased contribution of PICs to motoneuron firing.
- These results demonstrate that muscle vibration has the potential to influence the effects of neuromodulation on motoneuron firing. The potential of using vibration as a non-pharmacological neuromodulatory intervention should be further investigated.

Introduction

The likelihood of a motoneuron reaching the voltage threshold to generate an action potential depends on the cumulative effects of various types of descending and afferent ionotropic inputs, both inhibitory and excitatory. Importantly, motoneuron firing patterns are also greatly influenced by neuromodulatory inputs (Heckman et al., 2009). This non-linearity is primarily conferred by the activation of persistent inward currents (PICs) of positively charged ions in voltage-dependent channels predominantly located on the motoneuron dendrites. PICs amplify and prolong the effects of synaptic input (Heckmann et al., 2005), playing thereby an important role in motoneuron discharge characteristics. The contribution of PICs to motoneuron firing is proportional to descending monoaminergic inputs (i.e. serotonin and noradrenaline neuromodulators released by axons from the brainstem onto spinal motoneurons) (Heckmann et al., 2005). These descending neuromodulatory inputs are thought to be enhanced by an increase in voluntary drive (Orssatto, Mackay et al., 2021) and arousal state (Jacobs et al., 2002; Mesquita et al., 2022; Valentino & Van Bockstaele, 2008). Moreover, synaptic inputs from muscle afferents may alter neuromodulatory inputs by increasing the responsiveness of caudal raphe serotonergic neurons (Moolenaar et al., 1976), or by modulating descending corticospinal drive (McCloskey et al., 1974). The examination of non-pharmacological interventions that could modulate the effects of neuromodulatory inputs on PIC contribution to motoneuron firing may offer insight into and have implications for motoneuron function in healthy populations (Trajano et al., 2020), ageing (Orssatto, Borg et al., 2021) or clinical conditions (D'Amico, Murray et al., 2013).

Local vibration (LV) of a muscle or one of its tendons is one technique that could influence PICs. LV imposes repetitive muscle length changes and therefore provides a powerful stimulus to activate Ia afferents originating from muscle spindles (Burke et al., 1976b). One could argue that LV could induce stronger PIC activity given that LV reflexively activates motoneurons via Ia excitatory inputs,

increasing force output when applied during low-intensity contractions (Spiliopoulou et al., 2012), triggering the opening of PIC channels (Gorassini et al., 1998; Mesquita et al., 2021) and potentially increasing the responsiveness of caudal raphe serotonergic neurons (Moolenaar et al., 1976). However, ongoing LV during a ramp contraction could likely lead to less voluntary drive to reach a given level of force. Given that both serotonergic (Veasey et al., 1995) and noradrenergic (Noga et al., 2017) inputs might increase during voluntary contractions, with contribution of PICs to motoneuron firing likely being proportional to the level of voluntary drive (Orssatto, Mackay et al., 2021), decreasing the voluntary drive with assistance of LV-induced reflexive inputs could reduce the contribution of PICs to motoneuron firing.

Prolonged LV exposure may also influence the contribution of PICs to motoneuron firing during subsequent muscle contractions, as suggested by decreased motoneuronal output after LV exposure (Souron, Baudry et al., 2019; Souron, Besson, Millet et al., 2017). Accordingly, most LV studies investigating changes in force output following an acute bout (20–60 min duration) of LV exposure demonstrated a transient decrease in maximal voluntary force of the vibrated muscle group (for review see Souron, Besson, Millet et al., 2017). This has been largely suggested to result from the acute LV-induced decrease in spinal loop excitability after cessation of the vibration, as assessed by the H-reflex (Souron, Besson, Millet et al., 2017), which represents the monosynaptic excitation of motoneurons evoked by a single low-intensity electrical stimulus of Ia afferents. While increased presynaptic inhibition through primary afferent depolarisation has been commonly proposed as the most likely contributor to the H-reflex depression after prolonged LV, we recently provided novel mechanistic insight contradicting this hypothesis (Souron, Baudry et al., 2019). We demonstrated that LV induces a decrease in intrinsic motoneuron excitability, estimated by thoracic electrical stimulation (Souron, Baudry et al., 2019), which, unlike the H-reflex, is devoid of the classical presynaptic influence and considered a more appropriate method to assess the responsiveness of

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motoneurons to ionotropic synaptic input (McNeil et al., 2013). These results could partly explain the previously observed reduction in motor unit (MU) firing rates after prolonged LV exposure (Barrera-Curiel et al., 2019), likely underpinning maximal force decrement. While afferent inputs may increase caudal raphe serotonergic neuron responsiveness (Moolenaar et al., 1976), we previously speculated that prolonged LV could actually lead to decreased neuromodulatory inputs (Souron, Baudry et al., 2019), with a similar rationale to the one suggested in the context of exercise-induced fatigue (Taylor et al., 2016). Serotonergic neurons within the caudal raphe nuclei may present a decreased firing rate in response to prolonged activity, as observed in cats after prolonged treadmill locomotion (Fornal et al., 2006). This could then reduce the contribution of PICs to motoneuron firing, as further postulated by Skarabot et al. (2019). Yet such a hypothesis remains speculative and should be experimentally verified.

This study was designed to investigate the influence of LV on the contribution of PICs to firing hysteresis, which was estimated using the paired MU technique (Gorassini et al., 1998; Gorassini et al., 2002). First, we investigated the effect of ongoing LV on the contribution of PICs to tibialis anterior (TA) motoneuron firing. Then, to gain new insight into the mechanisms underlying the reduction of motoneuron output induced by prolonged LV, we examined the effect of prolonged LV on PICs. We hypothesised that both experiments would lead to lower contributions of PICs to motoneuron firing. Finally, voluntary contractions at different intensities were examined as the effects of PIC modulation might differ between motoneurons of different recruitment thresholds (Mesquita et al., 2020) and/or be dependent on the magnitude of descending voluntary drive (Orsatto, Mackay et al., 2021).

Methods

Participants

Twenty males volunteered to participate (age: 30 ± 7 years, height: 177.9 ± 7.0 cm, body mass: 76.5 ± 12.2 kg). Participants were asked to avoid caffeine and alcohol consumption as well as to abstain from strenuous exercise 24 h prior to the testing session. Participants provided informed consent and this study conformed to the ethical standards set by the *Declaration of Helsinki*, except for registration in a database. The study was approved by the local research ethics committees (CPP SudEst I; 1 408 208-2015-A00036-43).

Design

Data collection was performed in a single session. Participants were seated on the custom-built chair

of a dynamometer, and all the measurements were performed on the right leg. After placement of the high-density electromyography (HD-EMG) grid on the TA, participants initially performed dorsiflexion, triangular contractions for familiarisation and warm-up purposes. They performed ramp contractions to low and moderate torque targets until they were able to smoothly follow the torque path to the best of their abilities. Subsequently, participants were asked to perform at least two 3-s maximal isometric voluntary dorsiflexion contractions (MVCs) with a 60-s inter-trial passive rest. Additional MVCs were performed until the difference between the two best trials was less than 5%. Visual feedback of the torque trace was continuously provided on a screen in front of the participant, with standardised verbal encouragement. Participants then performed triangular isometric contractions without local vibration (CON-1), followed by triangular contractions with ongoing LV (VIB). After a 15-min resting period, participants performed similar triangular isometric contractions before (CON-2) and immediately after (POST-VIB) a 30-min LV exposure. At each time point, three triangular contractions were performed to both 20% and 50% of their MVC, with the ascending and descending phase lasting 10 s each (i.e. 2% of MVC/s and 5% of MVC/s, respectively). Triangular contractions to the same intensity were interspaced by 30 s, and contraction intensities were randomly ordered and separated by 1-min of rest.

Local vibration

During LV, the lower part of the TA (i.e. below the HD-EMG grid and above the TA distal tendon) was mechanically vibrated at 100 Hz (1-mm amplitude) by a vibrator (VB 115, Techno Concept, Manosque, France) fixated with a hook and loop fastener against the skin. LV with such characteristics is well known to represent a powerful stimulus for Ia afferents as those afferents are sensitive to small vibration amplitudes and fire synchronously with vibration frequencies up to 80–120 Hz (Roll & Vedel, 1982). LV was applied while the participants remained relaxed. The vibrator remained attached during the whole experiment and was only switched on during VIB trials (i.e. from 5 s before the beginning of the ramp contractions until the end of the contractions) and during the 30-min LV period.

Torque and electromyographic recordings

Dorsiflexion torque was measured during voluntary contractions by a calibrated instrumented pedal (CS1060 300 Nm; FGP Sensors, Les Clayes Sous Bois, France). Participants were seated upright in a custom-built chair with hips at 90° of flexion (0° = neutral position), right

knee at 120° of extension (180° = full extension) and right ankle in a neutral position. The foot was securely attached to the pedal with a custom-made hook and loop fastener. During all measurements, participants were provided with real-time feedback of the torque trace displayed on a large screen. The peak isometric dorsiflexion torque was taken as the highest value during the MVCs and used to set the intensity of the ramp contractions.

The skin under the electrodes was shaved, abraded with sandpaper and swabbed with alcohol. One flexible 64-electrode HD-EMG grid was placed on the TA muscle (13 rows × 5 columns). Electrodes (GR08MM1305; OT Bioelettronica, Turin, Italy) had a 1-mm diameter and 8-mm inter-electrode distance. The location of the TA was identified through palpation before the array was placed on the muscle belly, with the grid covering most of the TA proximal area (Del Vecchio et al., 2019). The array was attached to the skin by bi-adhesive foam and the skin-to-electrode contact optimised by filling the wells of the adhesive foam with conductive cream (AC Cream, Spes Medica, Genoa, Italy). Strap electrodes dampened with water were placed around the ankle (ground electrode) and wrist (reference electrode). HD-EMG signals were amplified (150×), collected in monopolar mode, through a 16-bit A/D (Quattrocento; OT Bioelettronica), band-pass filtered (10–500 Hz) and digitised at a rate of 5120 Hz. EMG signals were recorded and visualised using OTBioLab+ software (version 1.4.2.0, OT Bioelettronica) throughout the protocol to ensure acceptable signal quality.

Data analysis

Motor unit identification and tracking. HD-EMG signals and torque recordings were converted from the OT BioLab+ format into MATLAB-compatible data files (Version R2021B, The MathWorks, Natick, MA, USA). These files were then processed offline with the DEMUSE software tool (v5.01; The University of Maribor, Slovenia) that relies on the convolutive blind source separation method (Holobar & Zazula, 2007). Band-pass zero-phase (20–500 Hz), zero-phase second order finite impulse response high-pass differential (230 Hz) and notch (50 Hz and their higher harmonics) filters were applied in DEMUSE. For each subject, the three channels with the lowest signal-to-noise ratio were automatically removed to optimise decomposition, and 50 sequential decomposition runs were conducted in each ramp contraction independently. For each time point (CON-1, VIB, CON-2, POST-VIB) and contraction intensity (20% and 50%), only the best ramp contraction was retained for analysis. Ramps were selected by the experimenter based on smoothness and adherence to the torque template, as well as the number of identified MUs.

For each contraction intensity, the four selected ramps (i.e. CON-1, VIB, CON-2, POST-VIB) were concatenated and the same MUs were tentatively tracked over the four time points. MU duplicates were removed and spike trains visually inspected and manually edited by a trained investigator (Del Vecchio et al., 2020). Briefly, this process of careful inspection of the spike trains optimises the source separation procedure, excluding incorrectly identified firings and including missed firings. After editing, only MUs that presented a global pulse-to-noise ratio greater than 30 dB were retained for further analysis (Holobar et al., 2014).

Extraction of motor unit firing characteristics. After carefully editing the spike trains, firing events were converted into instantaneous firing rates and smoothed using a fifth order polynomial function, with additional MATLAB scripts and functions. All polynomials were visually inspected and if edge effects were observed at MU recruitment or derecruitment (i.e. a clear mismatch between the change in the smoothed and instantaneous firing rate), the MU from that specific trial was not included in further analyses. MU maximal firing rate was considered as the maximal value obtained from the polynomial curve, i.e. smoothed peak firing rate. Recruitment and derecruitment thresholds were also computed (i.e. torque level (% MVC) at the time when the MU started and stopped firing action potentials, respectively).

We then used the paired MU technique (Gorassini et al., 1998; Gorassini et al., 2002) to estimate the contribution of PICs to TA motoneuron firing (Fig. 1). This technique quantifies MU recruitment–derecruitment hysteresis (i.e. ΔF). Lower-threshold MUs (i.e. control units) were paired with higher-threshold MUs (i.e. test units), with the smoothed firing frequency of control units being used as an estimate of changes in the net synaptic input. In each MU pair, the hysteresis of the test unit was quantified by calculating the difference between the instantaneous smoothed firing rates of the control unit at recruitment and derecruitment of the test unit, which constitutes the ΔF (change in frequency) value (Gorassini et al., 2002). This score provides an estimation of the contribution of PICs to MU firing and has been validated by intracellular direct PIC measurements in animal models (Bennett, Li, Harvey et al., 2001) and in computer simulations (Powers & Heckman, 2015). A MU pair was only considered for analysis if the test unit was derecruited before the control unit. Furthermore, criteria were used to test the assumption that the control unit was a suitable proxy for net synaptic input. Pairs were included if rate-to-rate Pearson's correlation coefficients (r) between the smoothed firing rate polynomials of the test and control units (plotted at 5120 Hz in Microsoft Excel Version 2019) were >0.7 (Stephenson & Maluf, 2011),

so as to ensure that control and test MUs likely shared a common synaptic drive. The first 500 ms of the test unit were excluded from the correlation analysis to minimise contamination of a non-linear firing rate acceleration at the time of recruitment (Mottram et al., 2009). Moreover, only pairs with a recruitment time difference greater than 1 s were considered to meet the assumption that PICs in the control unit were fully activated when the test unit was recruited, avoiding the contamination of the aforementioned non-linear firing rate acceleration (Hassan et al., 2020). Finally, a saturation criterion was used, excluding pairs in which the control unit did not increase its firing rate more than 0.5 Hz after the recruitment of the test unit, as the control unit would not be sensitive to changes in synaptic drive (Stephenson & Maluf, 2011).

The quantification of the variables that were needed for the ΔF calculation, and the identification of suitable pairs and calculation of ΔF values were conducted in Microsoft Excel (Version 2019). Data were analysed in two

separate comparisons: CON-1 vs. VIB, to assess potential changes in the contribution of PICs to motoneuron firing (ΔF) during ongoing LV, and CON-2 vs. POST-VIB, to investigate the effects of prolonged LV on ΔF in a subsequent contraction. For each comparison, only pairs identified at the two considered time points were used for ΔF calculation. Importantly, ΔF s were calculated for individual test units as the average value obtained when the units were paired with multiple suitable control units, as previously conducted (Trajano et al., 2020).

Statistical analysis

Analyses of MU variables were conducted in R (version 4.0.5; R Foundation for Statistical Computing, Vienna, Austria), using RStudio environment (version 1.4.1106). Separate repeated-measures nested linear mixed-effects models were used to examine the effect of ongoing LV and prolonged LV on ΔF , MU recruitment threshold, MU derecruitment threshold, and peak smoothed firing

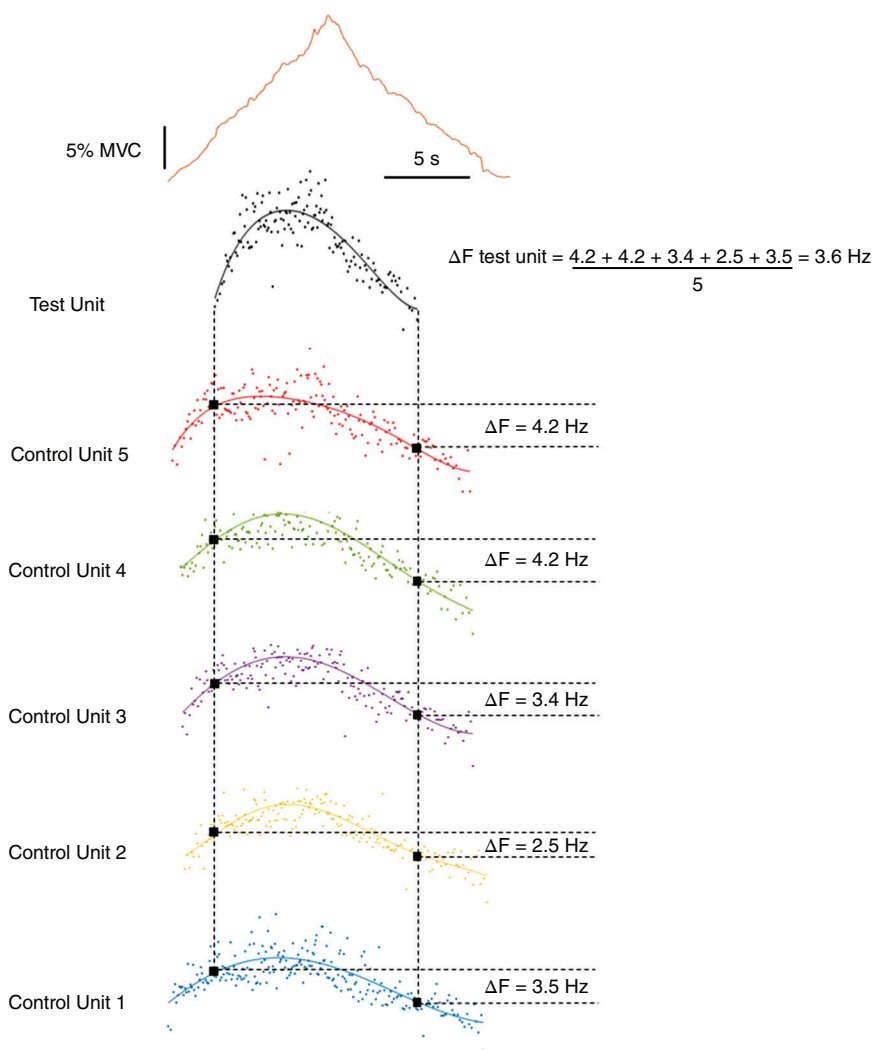


Figure 1. Calculation of ΔF from one test unit identified during a triangular contraction

Torque trace (orange, at the top) is shown during a 20-s ramp contraction up to 20% of maximal voluntary contraction (MVC). The instantaneous and smoothed (5th order polynomial fit) firing rate of six out of 30 motor units that were identified in this trial are shown to demonstrate the paired motor unit analysis approach. The test unit formed suitable pairs with five other control units. Thus, ΔF of the test unit was the average of ΔF scores computed when paired with those five control units. Dashed vertical lines indicate the time of recruitment and derecruitment of the test unit (recruitment thresholds of the six motor units represented here were 0.1, 1.0, 1.2, 1.2 and 1.3% MVC for control units and 6.0% MVC for the test unit). Dashed horizontal lines mark the smoothed firing rate of the control units at these times, and the difference between these two (ΔF) is indicated for each pair. [Colour figure can be viewed at wileyonlinelibrary.com]

rates of the whole sample of test units or MUs (Boccia et al., 2019). The *lmerTest* package was used (Kuznetsov et al., 2017). Variables were analysed with a random intercept (parallel slopes) model using 'time point' as a fixed effect, and 'participant' and 'test unit' (or 'MU') as random effects. After running the models, 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. In *post hoc* tests, estimated marginal means (with 95% confidence intervals (CI)) were quantified using the *emmeans* package (Lenth & Lenth, 2018).

Given the non-independence nature of the observations, repeated measures correlations were computed using the *rmcorr* package (Bakdash & Marusich, 2017) to investigate an association between both the changes in ΔF and MU recruitment threshold during the control time points. This allowed us to measure repeated measures correlation coefficients (r_{rm}). Correlation magnitude was interpreted based on Cohen's criteria (Cohen, 2013): trivial, $r_{rm} < 0.1$; weak, $r_{rm} = 0.1-0.3$; moderate, $r_{rm} = 0.3-0.5$; large, $r_{rm} = 0.5-0.7$; very large, $r_{rm} = 0.7-0.9$; and nearly perfect, $r_{rm} > 0.9$.

Results

Motor unit identification

MUs could not be identified in 2 out of 20 participants. Additionally, MUs could not be identified during 20% MVC ramps in two additional participants, and during 50% MVC ramps in two others. Therefore, data presented below are from 16 participants for both 20 and 50% MVC ramps. A total of 260 (16.2 ± 9.4 per participant) and 256 (15.8 ± 8.6 per participant) MUs were tracked during 20 and 50% MVC ramps, respectively, across the four time points. Of those MUs, a total of 27 (1.7 ± 2.0 per participant) and 20 (1.3 ± 1.5 per participant) MUs exhibited edge effects and were excluded from analysis.

Effects of ongoing local vibration on ΔF

We were able to track 77 test units (4.8 ± 3.7 per participant) and 461 pairs (28.8 ± 47.5 per participant) during 20% MVC ramps. During 50% MVC ramps, we tracked 145 test units (9.1 ± 5.2 per participant) and 788 pairs (49.3 ± 64.7 per participant).

In VIB, there were significant decreases in ΔF for both 20% ($F_{(1,76)} = 29.2$; $P < 0.001$) and 50% MVC ramps ($F_{(1,144)} = 20.9$; $P < 0.001$) (Fig. 2). In 20% ramps, the estimated marginal means (95% CI) of ΔF in CON-1 and VIB were 4.9 (4.3, 5.6) Hz and 4.2 (3.6, 4.9) Hz, respectively, with a mean difference of -0.7 (-0.5 ,

-1.0) Hz. In 50% ramps, ΔF in CON-1 and VIB were 5.4 (4.7, 6.2) Hz and 5.0 (4.2, 5.7) Hz, respectively, with a mean difference of -0.5 (-0.3 , -0.7) Hz. While there was no significant correlation between changes in ΔF from CON-1 to VIB and recruitment threshold of test unit in the control condition for 20% MVC ramps ($r_{rm} = -0.22$ (-0.45 , 0.04); $P = 0.09$), a small correlation between these variables was observed for 50% MVC ramps ($r_{rm} = 0.20$ (0.03 , 0.36); $P = 0.02$) (Fig. 3).

Effects of ongoing local vibration on motor units' thresholds and discharge rate

When considering all identified MUs during 20% MVC ramp contractions, there was no change in recruitment thresholds ($F_{(1,233)} < 0.01$; -0.00 (-0.4 , 0.4)% MVC; $P = 0.99$), or derecruitment thresholds ($F_{(1,233)} = 0.28$;

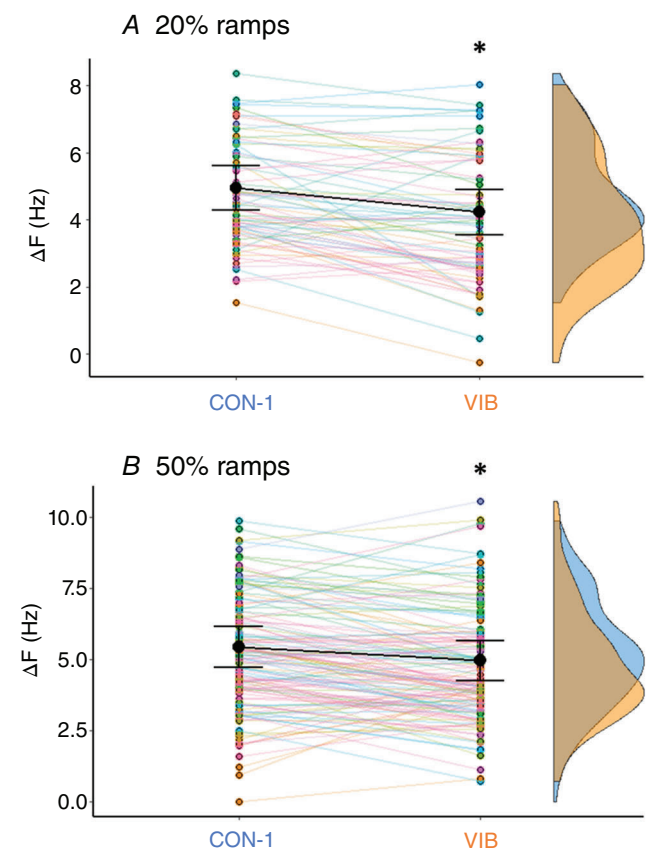


Figure 2. Changes in individual test unit ΔF values from control (CON-1) to vibration (VIB) trials in ramps performed to 20% (A, $n = 76$) and 50% (B, $n = 145$) of maximal voluntary torque

Each pair of points represents a test unit and each colour refers to one participant. Estimated marginal means are represented by black circles, with 95% confidence intervals. Kernel density estimation (density curves) of the data is represented on the right by half-violin plots (blue for CON-1 and orange for VIB). There was a significant decrease in ΔF in both 20% and 50% ramps ($*P < 0.001$). [Colour figure can be viewed at wileyonlinelibrary.com]

+0.08 (−0.2, 0.4)% MVC; $P = 0.60$) between CON-1 and VIB (Table 1). Yet, smoothed peak firing rates were significantly lower in VIB ($F_{(1,233)} = 48.1$; $P < 0.001$) with an estimated mean difference of −0.5 (−0.3, −0.6) Hz (Table 1). During 50% MVC ramp contractions, recruitment thresholds ($F_{(1,233)} = 9.9$; −0.9 (−0.3, −1.5)% MVC; $P = 0.002$), derecruitment thresholds ($F_{(1,233)} = 7.2$; −0.6 (−0.2, −1.1)% MVC; $P = 0.008$) and smoothed peak firing rates ($F_{(1,233)} = 4.1$; −0.2 (−0.005, −0.4) Hz; $P = 0.04$) were all significantly decreased in VIB (Table 1).

Effects of prolonged local vibration on ΔF

We tracked 89 test units (5.9 ± 3.6 per participant) and 512 pairs (34.1 ± 48.4 per participant) during 20% MVC ramps. During 50% MVC ramps, we tracked 136 test units (9.1 ± 5.3 per participant) and 624 pairs (41.6 ± 39.0 per participant). There were no significant changes in ΔF for either 20% ($F_{(1,88)} = 0.3$; $P = 0.58$) or 50% MVC ramps ($F_{(1,135)} = 0.40$; $P = 0.53$) after prolonged LV (Fig. 4). In 20% ramps, the estimated marginal means of ΔF in CON-2 and POST-VIB were 4.7 (4.0, 5.3) Hz and 4.6 (3.9, 5.2) Hz, respectively, with a mean difference of −0.1 (−0.4, 0.2) Hz. In 50% ramps, ΔF in CON-2 and POST-VIB were 5.4 (4.6, 6.2) Hz and 5.5 (4.7, 6.3) Hz, respectively, with a mean difference of +0.1 (−0.2, 0.3) Hz, respectively. However, a moderate correlation between changes in ΔF from CON-2 to POST-VIB and MU recruitment threshold of test units in the control

condition was observed in 50% MVC ramps ($r_{\text{rm}} = -0.40$ (−0.54, −0.24); $P < 0.001$) (Fig. 5B). A similar correlation, although smaller in magnitude, for 20% MVC ramp contractions was also observed ($r_{\text{rm}} = -0.27$ (−0.04, −0.47); $P = 0.02$) (Fig. 5A).

Effects of prolonged local vibration on motor units' thresholds and discharge rates

In POST-VIB, when considering all the identified MUs, recruitment thresholds ($F_{(1,233)} = 36.5$; +0.9 (0.6, 1.2)% MVC; $P < 0.001$), derecruitment thresholds ($F_{(1,233)} = 61.8$; +1.2 (0.9, 1.5)% MVC; $P < 0.001$) and smoothed peak firing rates ($F_{(1,233)} = 15.4$; −0.3 (−0.2, −0.5) Hz; $P = 0.001$) were all significantly different from CON-2 values (Table 2) during 20% MVC ramps. Similar results were observed during 50% MVC ramp contractions for recruitment thresholds ($F_{(1,233)} = 7.4$; +0.8 (0.2, 1.4)% MVC; $P = 0.007$), derecruitment thresholds ($F_{(1,233)} = 61.3$; +2.0 (1.5, 2.5)% MVC; $P < 0.001$) and smoothed peak firing rates ($F_{(1,233)} = 28.2$; −0.6 (−0.4, −0.8) Hz; $P < 0.001$) (Table 2).

Discussion

The main findings of the present study were that (1) assisting a voluntary contraction by reflexively recruiting motoneurons with concurrent LV reduces ΔF in TA motoneurons (Fig. 2), suggesting reduced contribution

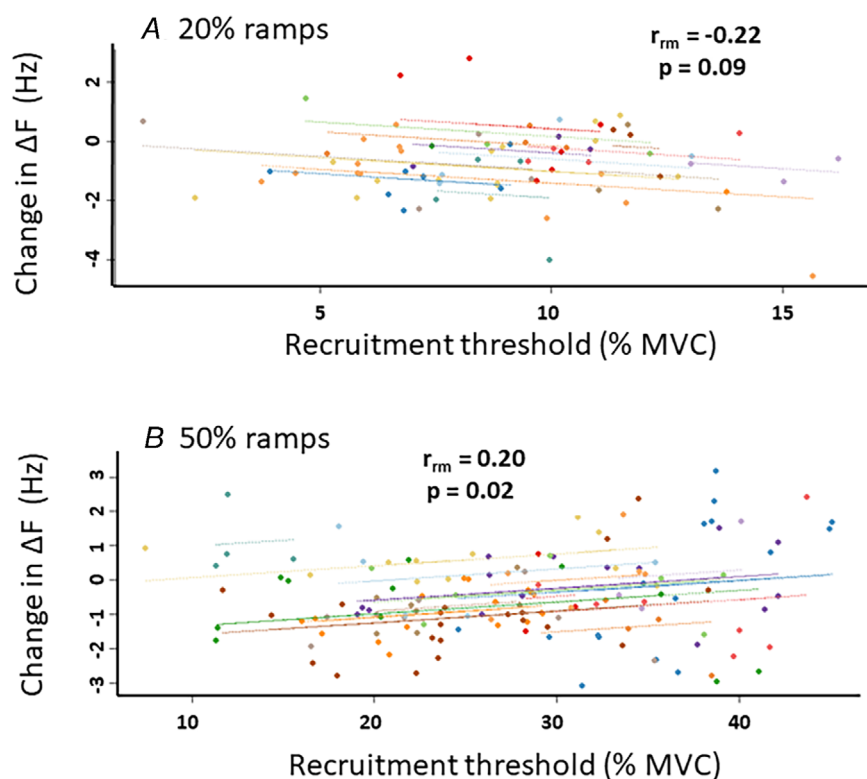


Figure 3. Repeated-measures correlation (r_{rm}) plots illustrating the association between recruitment threshold of test unit in the control trial and changes in ΔF in VIB

Upper panel (A) shows data from ramps performed to 20% of maximal voluntary contraction (MVC) torque and the lower panel (B) shows data from ramps performed to 50% MVC. Each colour represents a single participant and parallel lines are fitted to test units from each participant. The change in ΔF and the recruitment threshold of test units at control were not significantly correlated in the 20% MVC ramps. Nonetheless, a weak positive correlation in 50% MVC ramps suggests a more evident reduction of ΔF in motor units with a lower recruitment threshold. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1. Motor units' characteristics recorded during ramp contractions performed to 20 and 50% of maximal voluntary force before (CON-1) and during LV (VIB)

	CON-1	VIB	P
20%			
Recruitment threshold (% MVC)	6.7 (5.3, 8.2)	6.7 (5.3, 8.2)	0.99
Derecruitment threshold (% MVC)	6.7 (6.0, 7.5)	6.8 (6.0, 7.6)	0.60
Smoothed peak firing rate (Hz)	15.1 (14.2, 16.1)	14.6 (13.7, 15.6)	<0.001
50%			
Recruitment threshold (% MVC)	23.5 (19.8, 27.2)	22.6 (18.9, 26.3)	0.002
Derecruitment threshold (% MVC)	24.6 (20.8, 28.4)	23.9 (20.2, 27.7)	0.008
Smoothed peak firing rate (Hz)	20.1 (18.7, 21.5)	19.9 (18.5, 21.3)	0.04

Data are presented as estimated marginal means (95% confidence interval). *P*-values shown in bold indicate statistical significance.

of PICs to human motoneuron firing, and (2) the effects of prolonged LV on ΔF estimated during a subsequent contraction are possibly not uniform across the motoneuron pool (Fig. 5). The suggestion for the first time that contribution of PICs to motoneuron firing is weaker during ongoing, agonist LV demonstrates the potential to use LV to modulate the intrinsic excitability of motoneurons, likely by attenuating the magnitude of descending voluntary drive and neuromodulatory inputs. Furthermore, the absence of a clear effect of prolonged LV on PIC contribution to motoneuron firing with a possible differential effect across the motoneuron pool was unexpected and challenges interpretations of previous findings.

Effects of ongoing local vibration

Ongoing agonist LV decreased ΔF when participants were asked to follow a torque trace during an isometric ramp contraction. This suggests a reduced contribution of PICs to TA motoneuron firing. As LV induces excitatory Ia afferent input onto motoneurons (Burke et al., 1976a), an increased contribution of reflex activation to force output should be expected during ramp contractions with ongoing LV (Bongiovanni & Hagbarth, 1990; Gandevia, 2001; Macefield et al., 1993). Moreover, as (1) the opening of PIC channels can be triggered by excitatory Ia afferent input in humans (Gorassini et al., 1998; Mesquita et al., 2021) and (2) afferent inputs may increase the responsiveness of caudal raphe serotonergic neurons (Moolenaar et al., 1976), LV could lead to greater PICs for a given voluntary drive. Indeed, it was demonstrated that when participants have to maintain a constant level of effort (i.e. without feedback on force output), superimposing LV led to increased force output (Grande & Cafarelli, 2003; McCloskey et al., 1974; Spiliopoulou et al., 2012). In the present study, participants were rather instructed to follow a target force, and we postulate that a lower magnitude of corticospinal drive was required

to perform the task. This could have led to decreased monoaminergic inputs released onto motoneurons (i.e. serotonin (Veasey et al., 1995) and noradrenaline (Noga et al., 2017)), thereby limiting the contribution of PICs to motoneuron firing (Heckmann et al., 2005). This agrees with previous observations of lower ΔF scores in plantarflexor MUs during lower levels of voluntary descending drive (Orssatto, Mackay et al., 2021). As neuromodulatory inputs are known to amplify firing rates (Heckmann et al., 2005), it is expected that less neuromodulation would reduce peak firing rates. Accordingly, we observed a decreased smoothed peak firing rate during LV trials. Reduced firing rates could have led to earlier MU recruitment in order to achieve the required force, possibly explaining the decrease in recruitment and derecruitment thresholds observed in 50% MVC ramps. Alternatively, LV-induced inputs from Ia afferents could lead to the recruitment of a greater number of MUs contributing to the contraction, with those MUs not being identified by our decomposition methods. This reflexive recruitment during ongoing LV has been postulated by Grande & Cafarelli (2003) and would decrease the net synaptic input onto the identified MUs. Altogether, the present study provides indirect evidence, yet speculative, that lower levels of neuromodulation during a given level of voluntary force output can be achieved with extra reflexive inputs provided by LV, leading to decreased intrinsic motoneuron excitability.

Differential modulation might exist across the motoneuron pool in interventions that influence PICs (Mesquita et al., 2020). The present study used ramp contractions to both 20 and 50% MVC in order to assess any potential differential modulation of the contribution of PICs to firing hysteresis across the motoneuron pool. During 20% MVC ramps, no correlation was found between recruitment threshold and changes in ΔF , indicating that the reduction in ΔF was consistent across low to midrange recruitment thresholds. A similar reduction in ΔF across the pool of MUs identified during 20% MVC ramps has also been recently observed

during reciprocal inhibition and whole-body relaxation (Mesquita et al., 2022). Interestingly, a significant positive correlation between recruitment threshold and changes in ΔF during 50% ramps was found, with low to mid-range recruitment threshold MUs exhibiting a reduction in contribution of PICs to their firing in response to ongoing LV, whereas higher-threshold MUs were less susceptible to changes in ΔF . Potentially, the observed significant correlation in 50% MVC ramps would have been stronger if we had been able to identify a greater proportion of low-threshold MUs, a common limitation of HD-EMG during higher-intensity contractions (Farina et al., 2010; Holobar et al., 2009). Our findings support the hypothesis that larger motoneurons are less dependent on lasting PICs to maintain firing (Johnson et al., 2017; Lee & Heckman, 1998), making them less susceptible to reductions in neuromodulatory input. However, this

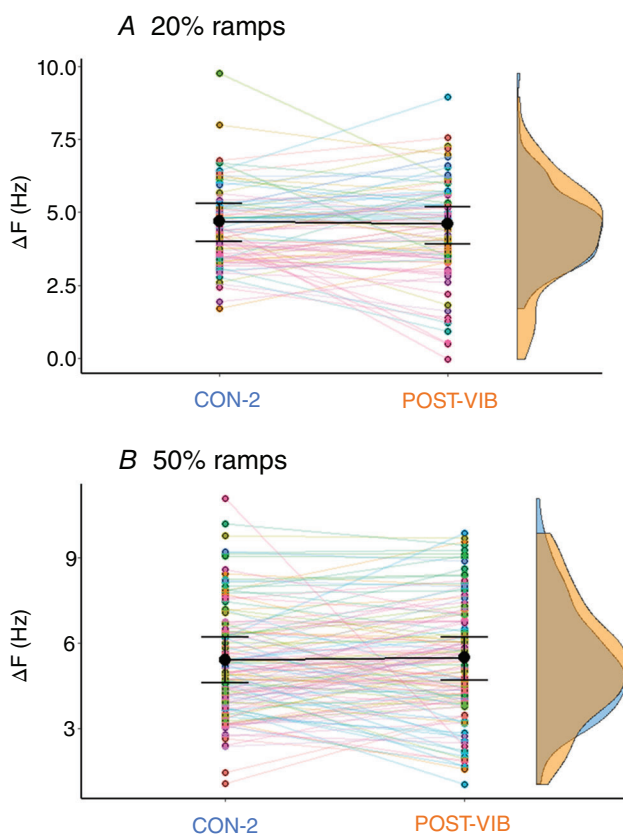


Figure 4. Changes in individual test unit ΔF values from control (CON-2) to post-vibration (POST-VIB) trials in ramps performed to 20% (A, $n = 89$) and 50% (B, $n = 136$) of maximal voluntary torque

Each pair of points represents a test unit and each colour refers to one participant. Estimated marginal means are represented by black circles, with 95% confidence intervals. Kernel density estimation (density curves) of the data is represented on the right by half-violin plots (blue for CON-2 and orange for POST-VIB). There was no difference between time points in either 20 or 50% ramps. [Colour figure can be viewed at wileyonlinelibrary.com]

speculation is based on previous findings of longer self-sustained firing in lower-threshold MUs after a brief excitatory synaptic input (Lee & Heckman, 1998). Thus, this hypothesis cannot be unequivocally applied to the magnitude of firing hysteresis during triangular contractions. Interestingly, some studies (Huh et al., 2017; Lee & Heckman, 1998; Stephenson & Maluf, 2011), but not all (Afsharipour et al., 2020; Mesquita et al., 2022), actually suggest stronger suprathreshold depolarising currents from PICs in higher-threshold MUs. An alternative underlying mechanism that could explain this differential effect of LV in different populations of motoneurons could be that the propagation of the vibratory stimulation along the muscle was progressively attenuated at higher contraction intensities, as this can decrease muscle spindle discharge (Burke et al., 1976a). This can be exacerbated by an insufficient gamma drive to the contracting muscle (Macefield, 2021), due to the relatively lower levels of corticospinal drive likely required during the ramps with concurrent LV. In agreement, a dependency of LV effects on the level of isometric strength has been previously observed (Spiliopoulou et al., 2012), showing that LV-induced Ia afferent inputs were ineffective in increasing force output during moderate (50% MVC), but not lower levels of force (10, 20, and 30% MVC).

Other than a reduction in corticospinal drive and neuromodulation, inhibitory mechanisms could explain the LV-induced alterations in MU characteristics observed in the present study. Greater levels of inhibition in TA motoneurons provide another feasible explanation. While neuromodulation strongly influences intrinsic motoneuron excitability, ionotropic synaptic inhibition can act as a significant opposing system, deactivating PICs (Heckman et al., 2009). During LV of a contracting muscle, the firing rate of Ib afferents from Golgi tendon organs has been reported to increase (Fallon & Macefield, 2007), which could have led to non-reciprocal inhibition of TA motoneurons. In addition, the classic Ia reciprocal inhibitory system seems to decrease the contribution of PICs to human motoneuron firing (Mesquita et al., 2022; Orssatto et al., 2022). As vibration stimuli might have led to activation of plantarflexor muscles Ia afferents to a certain extent (Katz et al., 1977), we cannot rule out the possibility that reciprocal inhibition onto TA motoneurons contributed to lower TA ΔF values and smoothed peak firing rates.

Effects of prolonged local vibration

Spinal motoneurons receive diffuse serotonergic innervation from the raphe nuclei (Alvarez et al., 1998; Bowker et al., 1981) and serotonin release is commonly triggered by motor activity (Veasey et al., 1995; Jacobs et al., 2002). Moolenaar et al. (1976) also demonstrated that this raphe-spinal pathway can be activated with a

variety of stimuli such as afferent inputs in response to electrical nerve stimulation. We previously hypothesised that prolonged activation of these serotonergic neurons by LV could lead to decreases in serotonin release from the brainstem (Souron, Baudry et al., 2019), as also observed after prolonged exercise (Fornal et al., 2006). With lower PICs, the motoneurons involved in muscle activation during the low-intensity contraction would likely have a more accentuated afterhyperpolarisation (Skarabot et al., 2019). This would lead to a decreased probability of reaching spiking threshold and generating an action potential upon the electrical stimulus. Moreover, the number of depolarised motoneurons upon stimulation could also be decreased due to a lower number of neighbouring *subliminal fringe* motoneurons near threshold (Lloyd, 1945) (see Fig. 1 of Skarabot et al. 2019). However, when considering the whole sample of test units, 30-min of LV had no effect on ΔF in the present study. We observed a differential effect of LV on MUs depending on their initial recruitment threshold (i.e. at CON-2). Changes in ΔF were negatively correlated with recruitment thresholds, spanning from a positive to a negative range (see Fig. 5). Although speculative, this could suggest a tendency for increased ΔF values in the early recruited MUs and for decreased ΔF values in the higher-threshold MUs. While future research should explore the mechanisms underlying this differential

effect on the contribution of PICs to motoneuron firing after prolonged LV as well as its potential implications, potential mechanisms involved in such differential changes in ΔF are proposed below.

Contrary to our hypothesis, and assuming that LV-induced afferent inputs actually reached the brainstem, the aforementioned correlation could be explained by increased serotonergic effects on excitatory 5-HT₂ receptors of lower-threshold motoneurons, increasing contribution of PICs to motoneuron firing (Harvey et al., 2006; Perrier & Hounsgaard, 2003). Repetitive activation of serotonergic neurons during the prolonged LV exposure (Moolenaar et al., 1976) could have led to enhanced serotonin availability during the subsequent ramp contraction and/or long-lasting facilitation of afferent inputs after 5-HT₂ agonist removal, as previously shown (Machacek et al., 2001; Shay et al., 2005). If this holds true, those early low-threshold MUs would have been recruited earlier and could have fired at a greater maximal rate, thereby increasing their contribution to force output. As low-threshold motoneurons are likely more dependent on lasting PICs to maintain firing (Lee & Heckman, 1998), a greater influence of neuromodulation in lower-threshold MUs could have occurred, with a consequently lower magnitude of voluntary drive onto higher-threshold MUs, decreasing their PIC-induced hysteresis (Fig. 4).

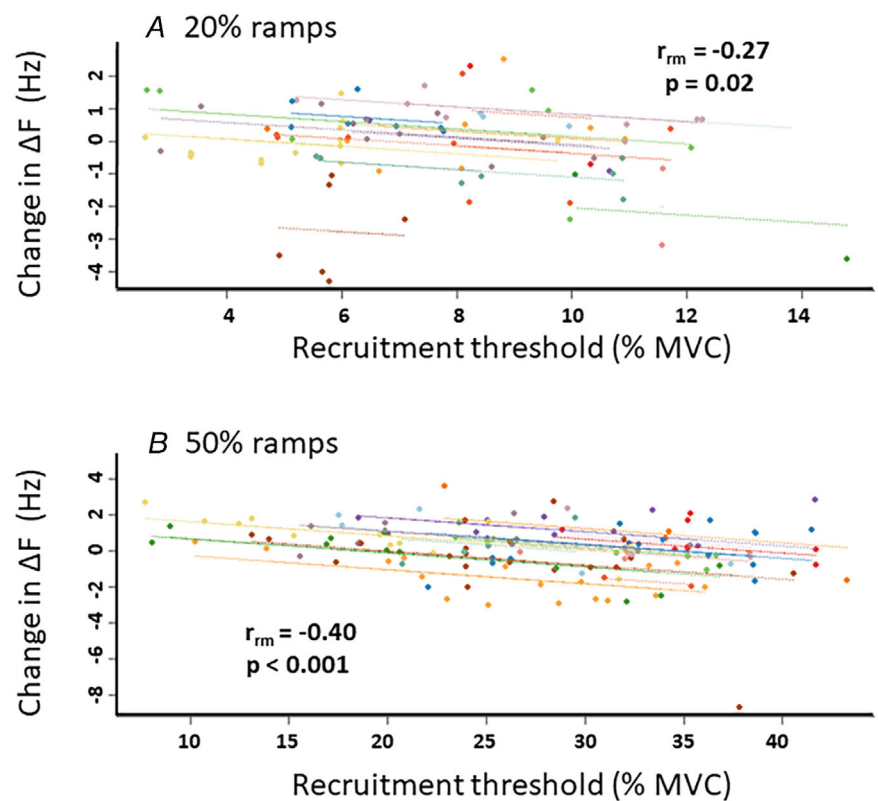


Figure 5. Repeated-measures correlation (r_{rm}) plots illustrating the association between recruitment threshold of test unit in the control trial and changes in ΔF in POST-VIB

Upper panel (A) shows data from ramps performed to 20% of maximal voluntary contraction (MVC) torque and the lower panel (B) shows data from ramps performed to 50% MVC. Each colour represents a single participant and parallel lines are fitted to test units from each participant. Weak and moderate correlations were observed in 20 and 50% MVC ramps, respectively, suggesting a tendency for positive changes in ΔF in lower-threshold motor units, while ΔF of higher-threshold units tended to decrease. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 2. Motor units' characteristics recorded during ramp contractions performed to 20 and 50% of maximal voluntary force before (CON-2) and after prolonged LV (POST-VIB)

	CON-2	POST-VIB	<i>P</i>
20%			
Recruitment threshold (% MVC)	6.3 (5.0, 7.6)	7.2 (5.9, 8.6)	<0.001
Derecruitment threshold (% MVC)	6.9 (6.2, 7.6)	8.1 (7.4, 8.8)	<0.001
Smoothed peak firing rate (Hz)	15.0 (14.1, 15.9)	14.7 (13.7, 15.6)	<0.001
50%			
Recruitment threshold (% MVC)	24.3 (20.6, 28.1)	25.1 (21.4, 28.9)	0.007
Derecruitment threshold (% MVC)	25.3 (21.8, 28.9)	27.3 (23.8, 30.9)	<0.001
Smoothed peak firing rate (Hz)	20.0 (18.6, 21.5)	19.7 (17.9, 20.9)	<0.001

Data are presented as estimated marginal means (95% confidence interval). *P*-values shown in bold indicate statistical significance.

Moreover, a neuromodulatory-induced increase in intrinsic motoneuron excitability could in fact lead to lower amplitudes of evoked responses (e.g. Crick & Wallis, 1991), due to occlusion or shunting of extra excitatory synaptic input. Contrary to our initial hypothesis relating decreased PICs to decreased responses to thoracic electrical stimulation (Skarabot et al., 2019; Souron, Baudry et al., 2019), a decrease in thoracic motor evoked potential due to increased PICs is also conceivable. Those evoked responses that we previously reported (Souron, Baudry et al., 2019) likely involved lower-threshold MUs because of the relatively small size of the evoked responses (approximately 10% of the maximal M-wave), and given that they were evoked during a low-intensity contraction (30% MVC). Greater contribution of PICs to motoneuron firing in lower-threshold MUs after prolonged LV exposure could attenuate the effects of superimposed electrically induced synaptic input because of an increased conductance (Bennett, Li, Siu et al., 2001) provided by PICs (i.e. higher likelihood of motoneurons firing in the tertiary range, marked by a lower sensitivity to firing modulation in response to extra excitatory synaptic input; Heckmann et al. 2005). This was clearly demonstrated by Fuglevand et al. (2015) in human MUs upon extra excitatory synaptic input provided by tendon vibration. It is then possible that motoneuron gain could have been paradoxically shallower after prolonged LV in our previous experiments (Souron, Baudry et al., 2019), making it more difficult to depolarise the lower-threshold motoneurons upon extra excitation of corticospinal axons (Heckman et al., 2005). Alternatively, decreases in H-reflex amplitude after prolonged LV in a relaxed state (Souron, Besson, Millet et al., 2017) could also be brought about by serotonergic-induced inhibition of group I primary afferent terminals (Proudfit & Anderson, 1974). D'Amico, Li et al. (2013) observed that activating inhibitory 5-HT₁ receptors on primary afferent terminals with a serotonin agonist decreases H-reflex amplitude in both people with and people without spinal cord injury.

It is not clear why we observed an increase in thresholds and a decrease in peak smoothed firing rates after prolonged LV. This modulation of MU parameters notionally fits with a delayed recruitment and activity of MUs to produce the required torque. Such results could partially support our hypothesis of lower-threshold MUs being recruited earlier after prolonged LV while higher-threshold MUs would be recruited later. For instance, the difficulty in identifying low-threshold MUs from surface electromyography (Farina et al., 2010; Holobar et al., 2009) could have led our results to be more influenced by changes in higher-threshold MUs demonstrating increased thresholds and decreased peak smoothed firing rate because of lower magnitude of voluntary drive (see above). Yet, no clear correlations between initial recruitment thresholds and LV-induced changes in thresholds or peak smoothed firing rates were observed (data not reported). Alternatively, it is possible that our intervention of prolonged LV increased muscle contractility in lower-threshold MUs which would be preferentially recruited during LV. Consequently, higher-threshold MUs, which were mostly identified by our decomposition methods, could have been recruited later and with lower firing rates during the subsequent ramp contractions. While LV-induced muscle contractility changes could be supported by some anecdotal findings of improved performance when LV was used as a warm-up modality (Cochrane, 2016a), this has not been confirmed by other studies (Cochrane, 2016b; Pamukoff et al., 2016; Souron, Zambelli et al., 2019). Moreover, it has been consistently reported that electrically elicited twitch amplitude and characteristics are unchanged after prolonged LV exposure (Souron, Besson, Millet et al., 2017).

The ability to produce strong contractions decreases after a single exposure to prolonged LV, with this phenomenon being shown in different muscle groups such as knee extensors (Souron, Besson, McNeil et al., 2017), plantarflexors (Ushiyama et al., 2005) and hand muscles

(Shinohara, 2005). Interestingly, MVC values remained unchanged after 30-min of LV on tibialis anterior in one study (Farabet et al., 2016), with this protocol being identical to the one performed in the current study. Even so, MVC values after prolonged LV were not assessed in the current study, and a change in relative (% MVC) forces during the ramps cannot be ruled out. Our results suggest a differential modulation of PICs across the motoneuron pool, which does not necessarily corroborate the hypothesis that changes in the ability to produce maximal force after prolonged LV are related to PIC activity. Nonetheless, the contribution of PICs to motoneuron firing was assessed during ramp contractions, and our findings cannot be directly generalised to PIC activity during maximal efforts. Estimation of PICs during MVCs remains an elusive goal and intracellular recordings of human motoneurons remain impossible. Moreover, the ΔF scores calculated in this study are an estimation of contribution of PICs to a specific motoneuron firing pattern – firing rate hysteresis or ‘PIC prolongation’. Thus, the effects of LV in this study cannot be directly extrapolated to other non-linearities in the motoneuronal input–output properties (Beauchamp et al., 2023; Binder et al., 2020) either, such as acceleration, saturation, warm-up and self-sustained firing. Other limitations of this methodological approach are that ΔF scores cannot estimate the contribution of subthreshold PICs to motoneuron firing (Afsharipour et al., 2020), are generally confined to relatively higher threshold MUs (Beauchamp et al., 2023), and can be contaminated by spike frequency adaptation (Vandenberk & Kalmar, 2014) or by alterations in the firing rate profile of the control units. Finally, it remains unclear whether the magnitude of changes in ΔF reported during ramp contractions in this study has meaningful implications in motor control. Nonetheless, overall changes in ΔF as small as 0.58 Hz have recently been associated with large changes in peak firing rates and with moderate to very large changes in broad measures of motor function (Orsatto et al., 2023).

Conclusion

The present study shows that the contribution of PICs to motoneuron firing (ΔF) can be modulated by LV. First, we observed a decrease in ΔF in TA MUs when LV was applied during ramp contractions, which likely reflected a decreased descending drive in response to LV-related MU activation. This should in turn have decreased neuromodulatory inputs. Second, prolonged exposure to LV led to differential effects of lower- vs. higher-threshold MUs on the contribution of PICs to motoneuron firing, with lower-threshold MUs tending to present increased ΔF and higher-threshold MUs decreased ΔF . This observation is provocative, challenging the interpretation of previous

observations, and underlying mechanisms should then be investigated in future studies.

References

- Afsharipour, B., Manzur, N., Duchcherer, J., Fenrich, K. F., Thompson, C. K., Negro, F., Quinlan, K. A., Bennett, D. J., & Gorassini, M. A. (2020). Estimation of self-sustained activity produced by persistent inward currents using firing rate profiles of multiple motor units in humans. *Journal of Neurophysiology*, **124**(1), 63–85.
- Alvarez, F. J., Pearson, J. C., Harrington, D., Dewey, D., Torbeck, L., & Fyffe, R. E. (1998). Distribution of 5-hydroxytryptamine-immunoreactive boutons on alpha-motoneurons in the lumbar spinal cord of adult cats. *The Journal of Comparative Neurology*, **393**(1), 69–83.
- Bakdash, J. Z., & Marusich, L. R. (2017). Repeated measures correlation. *Frontiers in Psychology*, **8**, 456.
- Barrera-Curiel, A., Colquhoun, R. J., Hernandez-Sarabia, J. A., & DeFreitas, J. M. (2019). The effects of vibration-induced altered stretch reflex sensitivity on maximal motor unit firing properties. *Journal of Neurophysiology*, **121**(6), 2215–2221.
- Beauchamp, J. A., Pearcey, G. E. P., Khurram, O. U., Chardon, M., Wang, Y. C., Powers, R. K., Dewald, J. P. A., & Heckman, C. J. (2023). A geometric approach to quantifying the neuromodulatory effects of persistent inward currents on individual motor unit discharge patterns. *Journal of Neural Engineering*, **20**(1), 016034.
- Bennett, D. J., Li, Y., Harvey, P. J., & Gorassini, M. (2001). Evidence for plateau potentials in tail motoneurons of awake chronic spinal rats with spasticity. *Journal of Neurophysiology*, **86**(4), 1972–1982.
- Bennett, D. J., Li, Y., & Siu, M. (2001). Plateau potentials in sacrocaudal motoneurons of chronic spinal rats, recorded in vitro. *Journal of Neurophysiology*, **86**(4), 1955–1971.
- Binder, M. D., Powers, R. K., & Heckman, C. J. (2020). Non-linear input-output functions of motoneurons. *Physiology*, **35**(1), 31–39.
- Boccia, G., Martinez-Valdes, E., Negro, F., Rainoldi, A., & Falla, D. (2019). Motor unit discharge rate and the estimated synaptic input to the vasti muscles is higher in open compared with closed kinetic chain exercise. *Journal of Applied Physiology*, **127**(4), 950–958.
- Bongiovanni, L. G., & Hagbarth, K. E. (1990). Tonic vibration reflexes elicited during fatigue from maximal voluntary contractions in man. *The Journal of Physiology*, **423**(1), 1–14.
- Bowker, R. M., Westlund, K. N., & Coulter, J. D. (1981). Serotonergic projections to the spinal cord from the midbrain in the rat: An immunocytochemical and retrograde transport study. *Neuroscience Letters*, **24**(3), 221–226.
- Burke, D., Hagbarth, K. E., Lofstedt, L., & Wallin, B. G. (1976a). The responses of human muscle spindle endings to vibration during isometric contraction. *The Journal of Physiology*, **261**(3), 695–711.

- Burke, D., Hagbarth, K. E., Lofstedt, L., & Wallin, B. G. (1976b). The responses of human muscle spindle endings to vibration of non-contracting muscles. *The Journal of Physiology*, **261**(3), 673–693.
- Cochrane, D. J. (2016a). The acute effect of direct vibration on muscular power performance in master athletes. *International Journal of Sports Medicine*, **37**(2), 144–148.
- Cochrane, D. J. (2016b). Does muscular force of the upper body increase following acute, direct vibration? *International Journal of Sports Medicine*, **37**(7), 547–551.
- Cohen, J. (2013). *Statistical Power Analysis for the Behavioral Sciences*. 2nd Edition.
- Crick, H., & Wallis, D. I. (1991). Inhibition of reflex responses of neonate rat lumbar spinal cord by 5-hydroxytryptamine. *British Journal of Pharmacology*, **103**(3), 1769–1775.
- D'Amico, J. M., Li, Y., Bennett, D. J., & Gorassini, M. A. (2013). Reduction of spinal sensory transmission by facilitation of 5-HT_{1B/D} receptors in noninjured and spinal cord-injured humans. *Journal of Neurophysiology*, **109**(6), 1485–1493.
- D'Amico, J. M., Murray, K. C., Li, Y., Chan, K. M., Finlay, M. G., Bennett, D. J., & Gorassini, M. A. (2013). Constitutively active 5-HT₂/α₁ receptors facilitate muscle spasms after human spinal cord injury. *Journal of Neurophysiology*, **109**(6), 1473–1484.
- Del Vecchio, A., Holobar, A., Falla, D., Felici, F., Enoka, R. M., & Farina, D. (2020). Tutorial: Analysis of motor unit discharge characteristics from high-density surface EMG signals. *Journal of Electromyography and Kinesiology*, **53**, 102426.
- Del Vecchio, A., Negro, F., Holobar, A., Casolo, A., Folland, J. P., Felici, F., & Farina, D. (2019). You are as fast as your motor neurons: Speed of recruitment and maximal discharge of motor neurons determine the maximal rate of force development in humans. *The Journal of Physiology*, **597**(9), 2445–2456.
- Fallon, J. B., & Macefield, V. G. (2007). Vibration sensitivity of human muscle spindles and Golgi tendon organs. *Muscle & Nerve*, **36**(1), 21–29.
- Farabet, A., Souron, R., Millet, G. Y., & Lapole, T. (2016). Changes in tibialis anterior corticospinal properties after acute prolonged muscle vibration. *European Journal of Applied Physiology*, **116**(6), 1197–1205.
- Farina, D., Holobar, A., Merletti, R., & Enoka, R. M. (2010). Decoding the neural drive to muscles from the surface electromyogram. *Clinical Neurophysiology*, **121**(10), 1616–1623.
- Fornal, C. A., Martin-Cora, F. J., & Jacobs, B. L. (2006). “Fatigue” of medullary but not mesencephalic raphe serotonergic neurons during locomotion in cats. *Brain Research*, **1072**(1), 55–61.
- Fuglevand, A. J., Lester, R. A., & Johns, R. K. (2015). Distinguishing intrinsic from extrinsic factors underlying firing rate saturation in human motor units. *Journal of Neurophysiology*, **113**(5), 1310–1322.
- Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiological Reviews*, **81**(4), 1725–1789.
- Gorassini, M., Yang, J. F., Siu, M., & Bennett, D. J. (2002). Intrinsic activation of human motoneurons: Possible contribution to motor unit excitation. *Journal of Neurophysiology*, **87**(4), 1850–1858.
- Gorassini, M. A., Bennett, D. J., & Yang, J. F. (1998). Self-sustained firing of human motor units. *Neuroscience Letters*, **247**(1), 13–16.
- Grande, G., & Cafarelli, E. (2003). Ia Afferent input alters the recruitment thresholds and firing rates of single human motor units. *Experimental Brain Research*, **150**(4), 449–457.
- Harvey, P. J., Li, X., Li, Y., & Bennett, D. J. (2006). Endogenous monoamine receptor activation is essential for enabling persistent sodium currents and repetitive firing in rat spinal motoneurons. *Journal of Neurophysiology*, **96**(3), 1171–1186.
- Hassan, A., Thompson, C. K., Negro, F., Cummings, M., Powers, R. K., Heckman, C. J., Dewald, J. P. A., & McPherson, L. M. (2020). Impact of parameter selection on estimates of motoneuron excitability using paired motor unit analysis. *Journal of Neural Engineering*, **17**(1), 016063.
- Heckman, C. J., Mottram, C., Quinlan, K., Theiss, R., & Schuster, J. (2009). Motoneuron excitability: The importance of neuromodulatory inputs. *Clinical Neurophysiology*, **120**(12), 2040–2054.
- Heckmann, C. J., Gorassini, M. A., & Bennett, D. J. (2005). Persistent inward currents in motoneuron dendrites: Implications for motor output. *Muscle & Nerve*, **31**(2), 135–156.
- Holobar, A., Farina, D., Gazzoni, M., Merletti, R., & Zazula, D. (2009). Estimating motor unit discharge patterns from high-density surface electromyogram. *Clinical Neurophysiology*, **120**(3), 551–562.
- Holobar, A., Minetto, M. A., & Farina, D. (2014). Accurate identification of motor unit discharge patterns from high-density surface EMG and validation with a novel signal-based performance metric. *Journal of Neural Engineering*, **11**(1), 016008.
- Holobar, A., & Zazula, D. (2007). Multichannel blind source separation using convolution kernel compensation. *IEEE Transactions on Signal Processing*, **55**(9), 4487–4496.
- Huh, S., Siripuram, R., Lee, R. H., Turkin, V. V., O'Neill, D., Hamm, T. M., Heckman, C. J., & Manuel, M. (2017). PICs in motoneurons do not scale with the size of the animal: A possible mechanism for faster speed of muscle contraction in smaller species. *Journal of Neurophysiology*, **118**(1), 93–102.
- Jacobs, B. L., Martin-Cora, F. J., & Fornal, C. A. (2002). Activity of medullary serotonergic neurons in freely moving animals. *Brain Research Brain Research Reviews*, **40**(1–3), 45–52.
- Johnson, M. D., Thompson, C. K., Tysseling, V. M., Powers, R. K., & Heckman, C. J. (2017). The potential for understanding the synaptic organization of human motor commands via the firing patterns of motoneurons. *Journal of Neurophysiology*, **118**(1), 520–531.
- Katz, R., Morin, C., Pierrot-Deseilligny, E., & Hibino, R. (1977). Conditioning of H reflex by a preceding sub-threshold tendon reflex stimulus. *Journal of Neurology, Neurosurgery, and Psychiatry*, **40**(6), 575–580.

- Kuznetsov, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, **82**(13), 1–26.
- Lee, R. H., & Heckman, C. J. (1998). Bistability in spinal motoneurons in vivo: Systematic variations in rhythmic firing patterns. *Journal of Neurophysiology*, **80**(2), 572–582.
- Lenth, R., & Lenth, M. R. (2018). Package 'lsmeans'. *The American Statistician*, **34**, 216–221.
- Lloyd, D. P. (1945). On the relation between discharge zone and subliminal fringe in a motoneuron pool supplied by a homogeneous presynaptic pathway. *The Yale Journal of Biology and Medicine*, **18**(2), 117–121.
- Macefield, V. G. (2021). The roles of mechanoreceptors in muscle and skin in human proprioception. *Current Opinion in Physiology*, **21**, 48–56.
- Macefield, V. G., Gandevia, S. C., Bigland-Ritchie, B., Gorman, R. B., & Burke, D. (1993). The firing rates of human motoneurons voluntarily activated in the absence of muscle afferent feedback. *The Journal of Physiology*, **471**(1), 429–443.
- Machacek, D. W., Garraway, S. M., Shay, B. L., & Hochman, S. (2001). Serotonin 5-HT₂ receptor activation induces a long-lasting amplification of spinal reflex actions in the rat. *The Journal of Physiology*, **537**(1), 201–207.
- McCloskey, D., Ebeling, P., & Goodwin, G. (1974). Estimation of weights and tensions and apparent involvement of a “sense of effort”. *Experimental Neurology*, **42**(1), 220–232.
- McNeil, C. J., Butler, J. E., Taylor, J. L., & Gandevia, S. C. (2013). Testing the excitability of human motoneurons. *Frontiers in Human Neuroscience*, **7**, 152.
- Mesquita, R. N. O., Skarabot, J., & Pearcey, G. E. P. (2020). Low-threshold motor units can be a pain during experimental muscle pain. *The Journal of Physiology*, **598**(13), 2545–2547.
- Mesquita, R. N. O., Taylor, J. L., Kirk, B., & Blazevich, A. J. (2021). Involuntary sustained firing of plantar flexor motor neurones: Effect of electrical stimulation parameters during tendon vibration. *European Journal of Applied Physiology*, **121**(3), 881–891.
- Mesquita, R. N. O., Taylor, J. L., Trajano, G. S., Skarabot, J., Holobar, A., Goncalves, B. A. M., & Blazevich, A. J. (2022). Effects of reciprocal inhibition and whole-body relaxation on persistent inward currents estimated by two different methods. *The Journal of Physiology*, **600**(11), 2765–2787.
- Moolenaar, G. M., Holloway, J. A., & Trouth, C. O. (1976). Responses of caudal raphe neurons to peripheral somatic stimulation. *Experimental Neurology*, **53**(2), 304–313.
- Mottram, C. J., Suresh, N. L., Heckman, C. J., Gorassini, M. A., & Rymer, W. Z. (2009). Origins of abnormal excitability in biceps brachii motoneurons of spastic-parietic stroke survivors. *Journal of Neurophysiology*, **102**(4), 2026–2038.
- Noga, B. R., Turkson, R. P., Xie, S., Taberner, A., Pinzon, A., & Hentall, I. D. (2017). Monoamine release in the cat lumbar spinal cord during fictive locomotion evoked by the mesencephalic locomotor region. *Frontiers in Neural Circuits*, **11**, 59.
- Orssatto, L. B. R., Borg, D. N., Blazevich, A. J., Sakugawa, R. L., Shield, A. J., & Trajano, G. S. (2021). Intrinsic motoneuron excitability is reduced in soleus and tibialis anterior of older adults. *Geroscience*, **43**(6), 2719–2735.
- Orssatto, L. B. R., Fernandes, G. L., Blazevich, A. J., & Trajano, G. S. (2022). Facilitation-inhibition control of motor neuronal persistent inward currents in young and older adults. *The Journal of Physiology*, **600**(23), 5101–5117.
- Orssatto, L. B. R., Mackay, K., Shield, A. J., Sakugawa, R. L., Blazevich, A. J., & Trajano, G. S. (2021). Estimates of persistent inward currents increase with the level of voluntary drive in low-threshold motor units of plantar flexor muscles. *Journal of Neurophysiology*, **125**(5), 1746–1754.
- Orssatto, L. B. R., Rodrigues, P., Mackay, K., Blazevich, A. J., Borg, D. N., R. S. T., Sakugawa, R. L., Shield, A., & Trajano, G. (2023). Intrinsic motor neurone excitability is increased after resistance training in older adults. *Journal of Neurophysiology*, **129**, 635–650.
- Pamukoff, D. N., Pietrosimone, B., Lewek, M. D., Ryan, E. D., Weinhold, P. S., Lee, D. R., & Blackburn, J. T. (2016). Immediate effect of vibratory stimuli on quadriceps function in healthy adults. *Muscle & Nerve*, **54**(3), 469–478.
- Perrier, J. F., & Hounsgaard, J. (2003). 5-HT₂ receptors promote plateau potentials in turtle spinal motoneurons by facilitating an L-type calcium current. *Journal of Neurophysiology*, **89**(2), 954–959.
- Powers, R. K., & Heckman, C. J. (2015). Contribution of intrinsic motoneuron properties to discharge hysteresis and its estimation based on paired motor unit recordings: A simulation study. *Journal of Neurophysiology*, **114**(1), 184–198.
- Proudfit, H. K., & Anderson, E. G. (1974). New long latency bulbospinal evoked potentials blocked by serotonin antagonists. *Brain Research*, **65**(3), 542–546.
- Roll, J. P., & Vedel, J. P. (1982). Kinaesthetic role of muscle afferents in man, studied by tendon vibration and micro-neurography. *Experimental Brain Research*, **47**(2), 177–190.
- Shay, B. L., Sawchuk, M., Machacek, D. W., & Hochman, S. (2005). Serotonin 5-HT₂ receptors induce a long-lasting facilitation of spinal reflexes independent of ionotropic receptor activity. *Journal of Neurophysiology*, **94**(4), 2867–2877.
- Shinohara, M. (2005). Effects of prolonged vibration on motor unit activity and motor performance. *Medicine and Science in Sports and Exercise*, **37**(12), 2120–2125.
- Skarabot, J., Mesquita, R. N. O., & Ansdell, P. (2019). Elucidating the neurophysiology of local vibration: Changes in neuromodulatory drive rather than presynaptic inhibition? *The Journal of Physiology*, **597**(24), 5753–5755.
- Souron, R., Baudry, S., Millet, G. Y., & Lapole, T. (2019). Vibration-induced depression in spinal loop excitability revisited. *The Journal of Physiology*, **597**(21), 5179–5193.
- Souron, R., Besson, T., McNeil, C. J., Lapole, T., & Millet, G. Y. (2017). An acute exposure to muscle vibration decreases knee extensors force production and modulates associated central nervous system excitability. *Frontiers in Human Neuroscience*, **11**, 519.
- Souron, R., Besson, T., Millet, G. Y., & Lapole, T. (2017). Acute and chronic neuromuscular adaptations to local vibration training. *European Journal of Applied Physiology*, **117**(10), 1939–1964.

- Souron, R., Zambelli, A., Espeit, L., Besson, T., Cochrane, D. J., & Lapole, T. (2019). Active versus local vibration warm-up effects on knee extensors stiffness and neuromuscular performance of healthy young males. *Journal of Science and Medicine in Sport*, **22**(2), 206–211.
- Spiliopoulou, S. I., Amiridis, I. G., Hatzitaki, V., Patikas, D., & Kellis, E. (2012). Tendon vibration during submaximal isometric strength and postural tasks. *European Journal of Applied Physiology*, **112**(11), 3807–3817.
- Stephenson, J. L., & Maluf, K. S. (2011). Dependence of the paired motor unit analysis on motor unit discharge characteristics in the human tibialis anterior muscle. *Journal of Neuroscience Methods*, **198**(1), 84–92.
- Taylor, J. L., Amann, M., Duchateau, J., Meeusen, R., & Rice, C. L. (2016). Neural contributions to muscle fatigue: From the brain to the muscle and back again. *Medicine and Science in Sports and Exercise*, **48**(11), 2294–2306.
- Trajano, G. S., Taylor, J. L., Orssatto, L. B. R., McNulty, C. R., & Blazeovich, A. J. (2020). Passive muscle stretching reduces estimates of persistent inward current strength in soleus motor units. *Journal of Experimental Biology*, **223**(21), jeb229922.
- Ushiyama, J., Masani, K., Kouzaki, M., Kanehisa, H., & Fukunaga, T. (2005). Difference in aftereffects following prolonged Achilles tendon vibration on muscle activity during maximal voluntary contraction among plantar flexor synergists. *Journal of Applied Physiology*, **98**(4), 1427–1433.
- Valentino, R. J., & Van Bockstaele, E. (2008). Convergent regulation of locus coeruleus activity as an adaptive response to stress. *European Journal of Pharmacology*, **583**(2–3), 194–203.
- Vandenberk, M. S., & Kalmar, J. M. (2014). An evaluation of paired motor unit estimates of persistent inward current in human motoneurons. *Journal of Neurophysiology*, **111**(9), 1877–1884.
- Veasey, S. C., Fornal, C. A., Metzler, C. W., & Jacobs, B. L. (1995). Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. *Journal of Neuroscience*, **15**(7), 5346–5359.

Additional information

Data availability statement

Individual data that support the findings of this study are available from the corresponding author on request.

Competing interests

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

Author contributions

T.L., R.N.O.M., C.G.B. and V.R. conceived and designed the research; T.L., C.G.B. and V.R. performed experiments; T.L., R.N.O.M., C.G.B. and V.R. analysed data; T.L., R.N.O.M., S.B., R.S., C.G.B. and V.R. interpreted the results of experiments; T.L. and R.N.O. prepared figures; T.L. and R.N.O.M. drafted the manuscript; T.L., R.N.O.M., S.B., R.S., C.G.B. and V.R. edited and revised the manuscript. All authors approved the final version of the manuscript and agreed 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.

Funding

This work was funded by the Jean Monnet University of Saint-Etienne through funding of the high-density electromyography device, and allowing a 6-month teaching discharge to T.L. to work on this study.

Acknowledgements

The authors thank Franck Le Mat and Dr Eleanor O'Brien for their assistance on statistical analysis.

Keywords

high-density electromyography, Ia afferents, motoneuron excitability, muscle spindles, neuromodulation

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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