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CONTRAÇÃO ISOMÉTRICA DE PREENSÃO MANUAL AUMENTA A EXCITABILIDADE INTRÍNSECA DO MOTONEURÔNIO DO TIBIAL ANTERIOR DE FORMA DOSE-DEPENDENTE

Lucas Campos Ugliara

BRASÍLIA
2025

CONTRAÇÃO ISOMÉTRICA DE PREENSÃO MANUAL AUMENTA A EXCITABILIDADE INTRÍNSECA DO MOTONEURÔNIO DO TIBIAL ANTERIOR DE FORMA DOSE-DEPENDENTE

Isometric handgrip contraction increases tibialis anterior intrinsic motoneuron excitability in a dose-dependent manner

LUCAS CAMPOS UGLIARA

Tese apresentada à Faculdade de Educação Física da Universidade de Brasília, como requisito parcial para obtenção do grau de Doutor em Educação Física.

Orientador: Dr. Amilton Vieira

Coorientador: Dr. Gabriel Siqueira Trajano

BANCA EXAMINADORA

Dr. Amilton Vieira (Presidente)

Universidade de Brasília

Dr. Anthony J Blazevich (Examinador externo)

Edith Cowan University

Dr. Matheus Machado Gomes (Examinador externo)

Universidade de São Paulo

Dr. Martim Francisco Bottaro Marques (Examinador interno)

Universidade de Brasília

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2025

DEDICATÓRIA

Aos meus pais, Altimar e Rosilda, por me ajudarem a entender meu verdadeiro propósito.

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LIST OF ACRONYMS, ABBREVIATIONS AND SYMBOLS

%	Percentage
%rTri	percentage of the right triangle
5-HT	Serotonin or 5-hydroxytryptamine
α	Significance level
β	Regression coefficient
Ca^{2+}	Calcium ion
CI	Confidence interval
CKC	Convolutive Kernel Compensation
d	Cohen's d
emmeans	Estimated marginal means (R package)
GPCR	G-protein coupled receptor
HDsEMG	High-density surface electromyography
MU	Motor unit
MVC	Maximum voluntary contraction
MVT	Maximum voluntary torque
N	Sample size or Newton
N·s	Newton·second
Na^{+}	Sodium ion
NE	norepinephrine or noradrenaline
p	p-value
PIC	persistent inward current
PMU	paired motor unit
pps	pulses per second
pps/%MVT	pulses per second per %MVT
r	Pearson correlation coefficient
robustlmm	robust linear mixed models (R package)
SE	standard error

σ	population standard deviation
SSRIs	selective serotonin reuptake inhibitors
SVR	Support Vector Regression
t	t-statistic
Δ	delta / change
ΔF	delta frequency

RESUMO

A contribuição de correntes internas persistentes (PICs) no disparo de motoneurônios no membro inferior tipicamente aumenta após uma contração remota de preensão manual, o que se acredita resultar de aumentos difusos no input serotoninérgico na medula espinhal. Investigamos se a intensidade, a duração e/ou o impulso da contração de preensão manual afetariam as estimativas de PICs em unidades motoras do músculo tibial anterior. Eletromiogramas de canais múltiplos foram adquiridos do tibial anterior de 21 participantes (18-40 anos), durante dorsiflexões a 20% do torque máximo individual, antes e depois de quatro condições de preensão manual: i) 80%15s, 80% de sua força máxima de preensão manual sustentada por 15s; ii) 40%15s, 40% sustentada por 15s; iii) 40%30s, 40% sustentada por 30s; e iv) Controle (sem preensão manual). A contribuição das PICs para o disparo auto-sustentado dos motoneurônios foi estimada através do delta da frequência (ΔF) utilizando a análise de unidade motora pareada. A 'altura do suporte', normalizada como percentagem de um triângulo retângulo (%rTri), foi utilizada para estimar os efeitos das PICs sobre a não-linearidade dos padrões de disparo, representando o drive neuromodulatório (regulação metabotrópica da excitabilidade do motoneurônio). O ΔF aumentou em 0,33 pulsos por segundo (pps; 95%IC 0,16–0,49, d=0,47) após a condição 40%30s e em 0,24 pps (0,09–0,38, d=0,34) após a 80%15s, mas permaneceu inalterada após a 40%15s e Controle. Semelhantemente, a altura do suporte aumentou em 2,24 %rTri (0,18–4,30, d=0,20) após a condição 40%30s e em 2,45 %rTri (0,64–4,25, d=0,22) após a 80%15s; permanecendo inalterada após a 40%15s e Controle. O aumento na contribuição das PICs para o disparo dos motoneurônios induzido por uma contração remota de preensão manual é impulso-dependente, em vez de da intensidade ou duração. Os aumentos paralelos no ΔF e na altura do suporte sugerem um input neuromodulatório aumentado na medula espinhal.

ABSTRACT

Persistent inward currents (PICs) contribution to motoneuron firing in the lower limb typically increase after a remote handgrip contraction, believed to result from diffuse increases of serotonergic input on the spinal cord. We investigated whether handgrip contraction intensity, duration, and/or impulse would affect estimates of PICs in tibialis anterior motor units. Multi-channel electromyograms were recorded from the tibialis anterior of 21 participants (18-40 years), during dorsiflexions at 20% of individual's maximal torque, before and after four handgrip conditions: i) 80%15s, 80% of their maximal handgrip strength sustained for 15s; ii) 40%15s, 40% sustained for 15s; iii) 40%30s, 40% sustained for 30s; and iv) Control (no handgrip). PICs contribution to self-sustained motoneuron firing was estimated with the delta frequency (ΔF) using the paired motor unit analysis. The 'brace height', normalised as a percentage of a right triangle (%rTri), was used to estimate the effects of PICs on the non-linearity of firing patterns, representing the neuromodulatory drive (metabotropic regulation of motoneuron excitability) onto the motoneurons. ΔF increased by 0.33 pulses per second (pps; 95%CI 0.16–0.49, d=0.47) after 40%30s and by 0.24 pps (0.09–0.38, d=0.34) after 80%15s but remained unchanged after 40%15s and Control. Similarly, brace height increased by 2.24 %rTri (0.18–4.30, d=0.20) after 40%30s and by 2.45 %rTri (0.64–4.25, d=0.22) after 80%15s; remaining unchanged after 40%15s and Control. The increase in PICs contribution to motoneuron firing induced by a remote handgrip contraction is impulse-dependent rather than intensity or duration. The parallel increases in ΔF and brace height suggest augmented neuromodulatory input onto the spinal cord.

Literature Review

Motoneurons over time

Spinal motoneurons are crucial for the proper function and control of our muscles, which are the primary structures enabling our interaction with the known world. Motoneurons are nerve cells formed by the soma, axon, and dendrites (Duchateau & Enoka, 2011). The concept of the motor unit was first raised in an experiment on the cutaneous dorsi of the frog by Keith Lucas, who observed that muscle twitch contractions gradually increased with the intensity of muscle nerve stimulus (Lucas, 1905, 1909). However, the term motor unit was first introduced by Charles S. Sherrington, who defined motoneurons as the "common final pathway" (Barbara & Clarac, 2011; Duchateau & Enoka, 2011; Sherrington, 1906). Later, the anatomical and physiological concept of motor unit (i.e., a single spinal motor neuron and its innervated muscle fibres) was formally proposed in the 1920s by Sherrington and Edward Liddell (Liddell & Sherrington, 1925). This way, the motor unit was understood as the fundamental functional endpoint for all descending motor commands, providing a direction for subsequent investigations and advancing our understanding of the motor-neural system.

Building upon Liddell and Sherrington's work, Henneman developed his size principle theory and developed the deterministic rule of motor unit recruitment, refining the understanding on how the Central Nervous System (CNS) regulates force: motor units are recruited in an orderly sequence from smallest (low-threshold, fatigue-resistant) to largest (high-threshold, powerful), allowing the motor system to perform from fine force to maximal force (Henneman et al., 1965b, 1965a; Henneman & Mendell, 1981; McPhedran et al., 1965; Wuerker et al., 1965). The size principle, when combined with the concept of a common drive, theoretically suggests a rigid, size-ordered control (Hug et al., 2023). However, subsequent studies demonstrated that the motoneuron is more than a passive receiver; its excitability and output are dynamically controlled by a combination of ionotropic inputs (excitatory and inhibitory neurotransmitters, such as glutamate and GABA/glycine, respectively) and neuromodulatory inputs (Marshall et al., 2022; Mesquita et al., 2024).

Neuromodulation

Neuromodulation is responsible for altering the motoneuron's intrinsic electrical properties and input-output function, enabling a wide range of flexible control and adaptability in motor unit behaviour (Mesquita et al., 2024). Although many different neurotransmitters are involved in neuromodulation (Slater et al., 2022), serotonin (5-HT) and norepinephrine (NE) are believed to elicit the strongest response by activating persistent inward currents (PICs) (Heckman, Johnson, et al., 2008; Heckman et al., 2009; Lee & Heckman, 1996). Motor function is likely to play a key role in 5-HT release in the spinal cord (Jacobs et al., 2002; Veasey et al., 1995), where the intensity of voluntary motor outflow influences the concentration of 5-HT released in the spinal cord (Jacobs et al., 2002). On the other hand, although evidence from experiments performed in the cat lumbar spinal cord suggests that both 5-HT and NE extracellular concentration increased during centrally-generated locomotion (Noga et al., 2017), NE release is primarily associated with the body's general state of stress and arousal (Ross & Van Bockstaele, 2021; Valentino & Van Bockstaele, 2008). Moreover, the spinal cord is densely innervated by serotonergic branches (Alvarez et al., 1998; Bowker et al., 1981), which diffusely project from the raphe nuclei to the spinal cord (Heckman, Hyngstrom, et al., 2008). Additionally, it is unclear whether NE also presents a similar diffuse descending pathway.

Excitatory inputs

5-HT and NE connect to receptors in motoneuron dendrites in the spinal cord and increase the chance of opening calcium (Ca^{2+}) and sodium (Na^{+}) channels, raising motoneurons' firing up to five-fold (Figure 1A and 1B) (Hounsgaard & Kiehn, 1993; Lee & Heckman, 1999, 2000). Computer simulations using a pool of 100 simulated motor units with properties based on the cat medial gastrocnemius motoneuron pool and muscle (Heckman & Binder, 1991) suggested that the absence of 5-HT and NE influence on motoneuron firing would dampen the maximal motor output in 60% (Figure 1D). Once released in the spinal cord, 5-HT and NE bind to G-protein coupled receptors (GPCRs), which activate a cascade of biochemical events that culminate in the opening of Ca^{2+} L-type and Na^{+} channels (Carlin et al. 2000; Lee & Heckman, 1999a; Li et al. 2004; Simon et al. 2003).

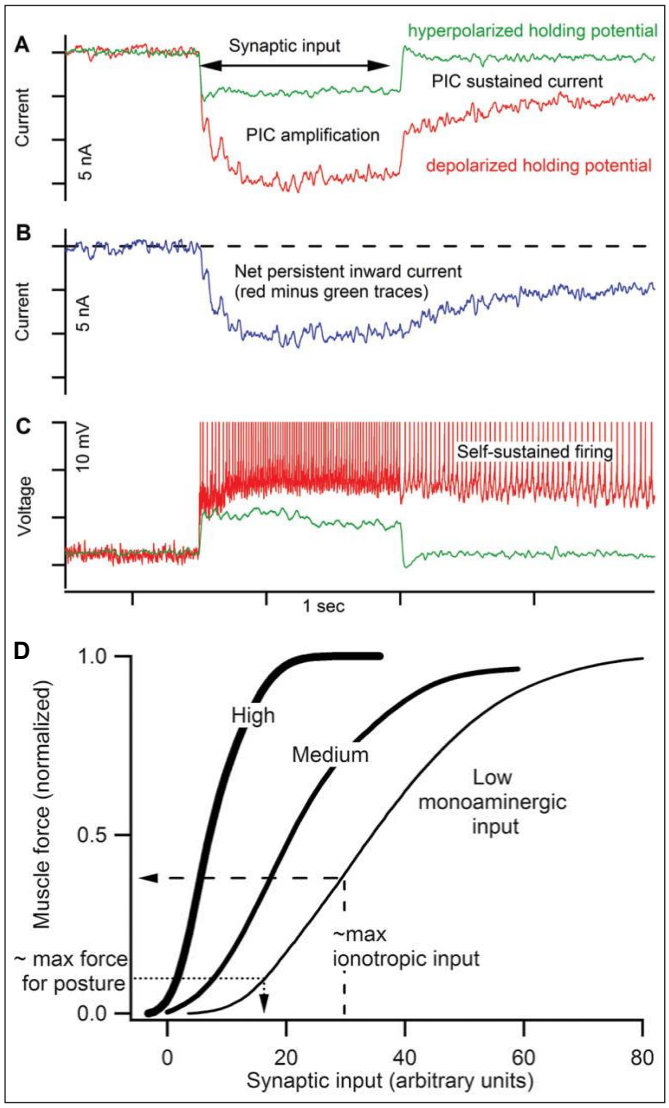

Figure 1. (from Heckman et al. (2008) with permission) Amplification and prolongation of synaptic input by the dendritic persistent inward current (PIC) in a low-threshold, type S motoneuron.

1A Steady synaptic input was generated by 1.5 seconds of high-frequency activation of a monosynaptic, ionotropic source, muscle spindle Ia afferents. At a hyperpolarized holding potential (–90 mV; green trace), this input produced a steady current with a sharp onset and offset. At depolarized holding potential (~–55 mV; red trace), the very same input is greatly amplified and prolonged by the PIC. Baseline holding currents are removed to allow the traces to be superimposed.

1B The difference between the currents in *A* reflects the net contribution of the dendritic PIC.

1C Representation of self-sustained firing (hysteresis) in an unclamped condition.

1D Visualization based on computer simulations using motor unit data from cat medial gastrocnemius to illustrate the effect of neuromodulatory input on input-output motor function.

Data are from Lee and Heckman (1996).

L-type Ca^{2+} channels exhibit slow activation during depolarisation but show little inactivation over time (Lee & Heckman, 1999; Svirskis & Hounsgaard, 1997). When activated, these channels generate a long-lasting influx of Ca^{2+} that contributes to a depolarised state that supports self-sustained firing even after synaptic input has ceased (Figure 1C) (Heckman, Johnson, et al., 2008; Lee & Heckman, 1999; Svirskis & Hounsgaard, 1997). The Ca^{2+} that enters through these channels can also bind to calmodulin, which interacts with the channel's pre-IQ domain to enhance its open probability further, thereby reinforcing current persistence (Binder et al., 2020). This slow but persistent activation forms plateau potentials, composing a mechanism for prolonged motor output and stable force generation. In parallel, voltage-gated Na^{+} channels activate rapidly in response to depolarisation but inactivate more gradually during sustained activity (Catterall, 1992; Hodgkin & Huxley, 1952). The transient sodium current contributes to the rapid rising phase of the action potential (Hodgkin &

Huxley, 1952), whereas a minor persistent component remains active during prolonged depolarisation. This persistent Na^+ current amplifies subthreshold synaptic inputs, reduces the activation threshold, and promotes repetitive firing by maintaining a depolarising drive throughout the interspike interval (Heckman et al., 2005; Kuo et al., 2006).

Recruitment de-recruitment hysteresis

The difference in kinetic behaviour of Ca^{2+} and Na^+ PICs results in nonlinear motoneuronal input-output relationships. This way, during a gradual increase and subsequent decrease in synaptic voluntary drive, we can distinguish three phases in motoneurons' firing that are a consequence of the characteristics of Ca2+ and Na+ channels (Figure 2). The initial phase lasts ~1 to 2 seconds. It is referred to as the secondary range of firing, characterised by a high-gain phase (i.e., small increases in synaptic input generate substantial changes in firing rate), indicating the progressive activation of PICs, likely mediated by Ca^{2+} currents (Lee & Heckman, 1998; Powers et al., 2012). The second phase is described as the tertiary firing range, when PICs approach full activation and the firing rate transitions from a high-gain to low-gain, rising toward a saturation or plateau (Heckman & Enoka, 2012). The third phase consists of the recruitment-de-recruitment hysteresis, where motoneurons continue firing at lower input levels than those required for recruitment, a phenomenon that demands sustained PIC activation, likely influenced by the slow deactivation of Ca^{2+}-mediated PICs (Hounsgaard et al., 1988; Bennett et al., 2001). Thus, these three phases of nonlinear firing characteristics (i.e., acceleration, saturation, and hysteresis) demonstrate the importance of both Ca2+ and Na+ PICs in modulating motoneuron excitability, synaptic integration, and the control of muscle force.

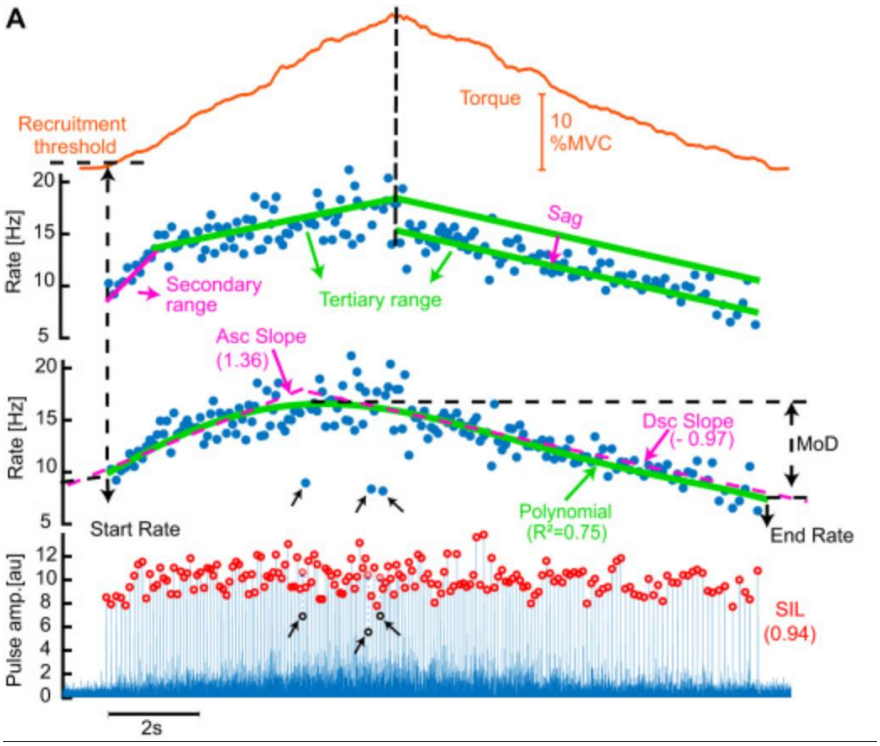

Figure 2. Representation of the different phases of the motoneuron firing pattern. From Afsharipour et al. (2020) with permission.

Parameters measured from the motor unit firing rate profile. A, top trace: torque profile for a 20% maximum voluntary contraction (MVC) showing recruitment threshold of the motor unit. Second trace, corrected firing rate profile (blue dots) of decomposed motor unit (see below for details) and straight line fit to the secondary (pink) and tertiary (green) firing range. Time of peak firing rate is marked by vertical dashed line and denotes start of lower offset (sag) in firing rate during the descending phase of the contraction. Third trace, uncorrected firing rate profile. The 5th-order polynomial line fit to the firing rate profile is marked with a green line where the coefficient of determination (R2) of the fit was measured. Straight-line fit to the data points on the entire ascending and descending portion of the polynomial line is marked with a dashed pink line from which the ascending (Asc) and descending (Dsc) slope values were measured, respectively. Black downward arrows mark the start and end firing rates measured from the polynomial line. Modulation depth (MoD) is the maximum rate minus the minimum rate measured from the polynomial line. Bottom trace, the train of pulse amplitudes (blue lines) from the decomposition algorithm (marking firing times of the decomposed motor unit) with an accuracy (silouette, SIL) value of 0.94. Red circles mark pulses selected by the blind source algorithm, and black circles mark pulses that were not selected, producing abnormally low firing rate values marked by small black arrows in the firing rate profile of the third trace. Dashed blue arrows point to the reestimated pulses (dotted red circles) following recomputation of the pulse train to include the missed pulses. The resulting corrected firing rate profile after the recalculation/reestimation is plotted in the second trace. Data are from participant 4M.

Inhibitory inputs

Another important characteristic of PICs is that they can be easily ceased by inhibitory input (Hounsgaard et al., 1988; Hultborn et al., 2003). For example, the in vivo voltage clamp technique was used to show that small stretching and shortening rotations (± 10°) of the cat ankle joint likely induced Ia reciprocal inhibition of the

antagonist muscle, resulting in ~50% reduced dendritic PICs ankle extensors motoneurons (Hyngstrom et al., 2007). More recently, studies have used local vibration of antagonist muscles to induce Ia reciprocal inhibition during voluntary ramp-shaped contractions and observed reduced estimations of PICs in the tibialis anterior of young adults (Lapole et al., 2023) and the soleus of both young and older adults (Orssatto et al., 2022). More recently, tibialis anterior motoneurons in young adults have shown decreased estimates of PICs following the co-contraction of ankle muscles (Gomes et al., 2024). Thus, neuromodulation is not solely the result of increased motoneuron excitability through neurotransmitter action, but also the result of interactions with inhibitory inputs. This excitatory-inhibitory interaction enables proper control during various muscular tasks, primarily due to the diffuse nature of the descending axons originating from the raphe nuclei and locus coeruleus.

Diffuse descending serotonin inputs

The 5-HT projections to the spinal cord are largely diffuse, following a path along the entire ventral white matter of the spinal cord (Proudfit & Clark, 1991; Skagerberg & Björklund, 1985). While this characteristic of the monoaminergic system has been extensively investigated in animal models through invasive techniques (Barreiro-Iglesias et al., 2015; Heckman, Hyngstrom, et al., 2008; Noga et al., 2017), technological limitations restrict direct measurement in humans. Specifically, invasive approaches, such as the voltage-clamp technique, are unsuitable, and the lack of available markers for the activation of raphe nuclei renders direct measurement of serotonin release onto spinal motoneurons impossible (Kavanagh & Taylor, 2022). Consequently, different non-invasive and indirect techniques have been developed to estimate motoneuron excitability and PICs in humans.

The Jendrassik manoeuvre, and derivations of this technique, is likely the most studied phenomenon of a remote contraction influencing increased responses in muscle groups not involved in the original task. First described in 1883 (Jendrassik, 1883), the Jendrassik manoeuvre involves instructing an evaluated patient to clench their teeth, then clasping their hands and pulling them apart while a hammer strikes the knee tendon (Figure 3). This is used to increase the knee extension reflex theoretically. Based on this principle, Ebben (2006) suggested in a comprehensive study that remote voluntary contraction, including that observed in the Jendrassik

manoeuvre, might elicit concurrent potentiation and help to increase human performance during sports and resistance training. In the sporting context, jaw clenching is a practical strategy for performing remote contraction and has been investigated by various research groups. Track and field athletes experienced an increased rate of force development and decreased time to peak force when performing a countermovement jump task with jaw clenching (Ebben et al., 2008). Also, both the maximal force and the rate of force development (0-50 ms, 50-100 ms, and 100-150 ms time windows) at the maximal isometric knee extension test increased when rugby athletes performed jaw clenching (Rizzato et al., 2024). Jaw clenching has also been shown to decrease the dislocation of the centre of gravity in healthy male participants (31.6 ± 8.51 years), both in the presence and absence of visual input, suggesting an improvement in postural stability (Alghadir et al., 2015). Furthermore, higher-intensity teeth clenching (>50% of maximal voluntary contraction) increased peak torque during an isometric dorsiflexion task and the soleus/tibialis anterior electromyography ratio (Hirabayashi et al., 2022). A recent systematic review suggested that using mouthguards during sports practice could be beneficial not only for protection, but also for their ergogenic effects, as observed in jump ability and knee extension movements (Miró et al., 2021). The jaw muscles (i.e., masseter, temporalis, medial pterygoid, and lateral pterygoid) are innervated by branches of the mandibular division (V3) of the trigeminal nerve (cranial nerve V), whose motor nucleus originates in the brainstem (see Figure 4) (Corcoran & Goldman, 2023). This suggests that the voluntary activation of a group of muscles might somehow elicit increased performance (usually greater force-generating capacity) in other muscular groups, with different nerve origins and that were not involved in this task. Using a similar logic, recent studies have also investigated this phenomenon between the upper and lower limbs, seeking evidence of an intersegmental facilitation mechanism (Figure 5). Moreover, recent advances in technology have enabled the investigation of mechanisms that may mediate this influence, such as the activation of PICs.

Figure 3. Arthur van Gehuchten performing the Jendrassik maneuver.

Figure 186 of Les Maladies nerveuses (Gehuchten, 1920). Original glass plate scanned and digitalized by the Museum voor Fotografie, Antwerp, Belgium. Permission: Public Domain due to age.

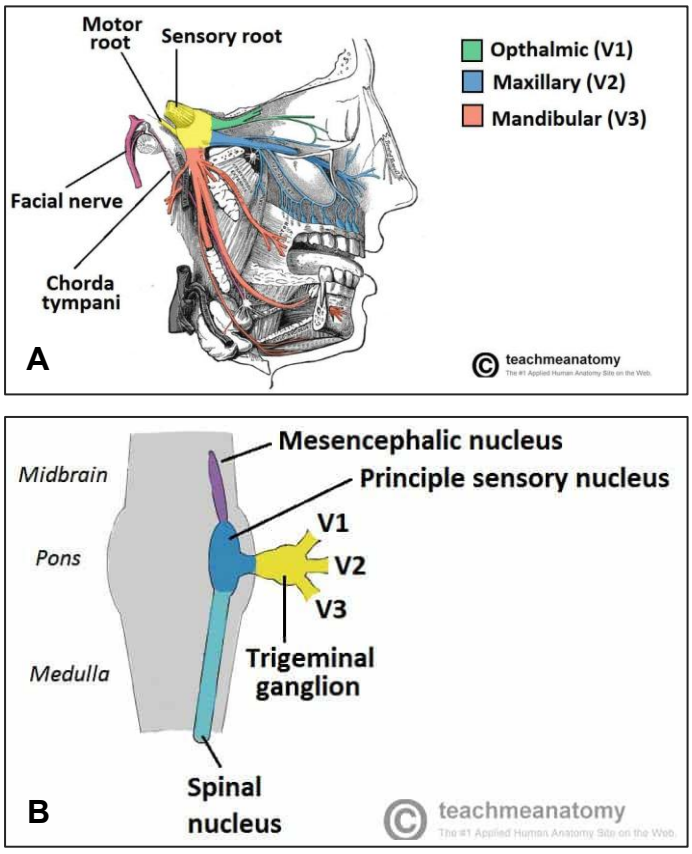

Figure 4. Innervation of jaw muscles (i.e., masseter, temporalis, medial pterygoid, and lateral pterygoid) by branches of the mandibular division (V3) of the trigeminal nerve (cranial nerve V), originated in the brainstem.

4A Overview of the deep distribution of the trigeminal nerve and its terminal branches.

4B The origin of the sensory aspect of the trigeminal nerve. Note that the nuclei are situated within in the CNS, and the ganglia outside the CNS.

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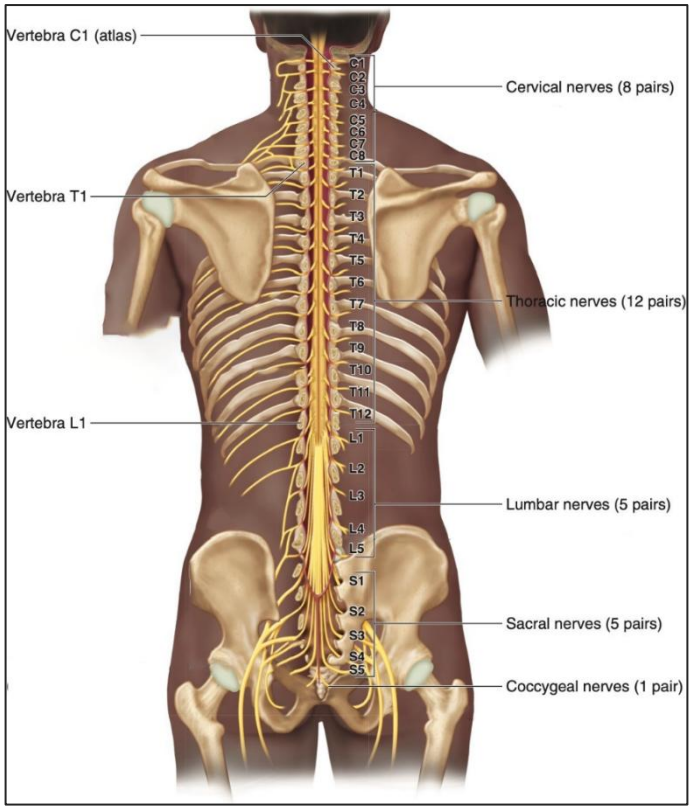

Figure 5. Overview of the origin and distribution of nerves responsible for handgrip contraction and dorsiflexion.

Handgrip muscles (i.e., flexor digitorum profundis, flexor pollicis longus, extensor digitorum communis, brachioradialis, thenar muscles, hypothenar muscles, interossei, and lumbricals) are innervated by cervical and thoracic nerves (C5 to T1). Dorsiflexion muscles (i.e., tibialis anterior, extensor digitorum longus, extensor hallucis longus, and peroneus tertius) are innervated by lumbar and sacral nerves (L4 to S1). The descending serotonin input extends throughout almost the entire spinal cord. Handgrip contractions theoretically increase serotonin concentration in the region of tibialis anterior motoneurons' dendrites. (Image credit: "Spinal Cord and Spinal Nerves" by Gabrielle Spurlock is licensed under CC BY-NC-SA 4.0, based on original by "KnowledgeWorks - Drawing Spinal nerves and plexuses - English labels" by KnowledgeWorks Global Ltd.)

Pioneering the investigation into the influence of remote contraction on human motoneuron excitability, Wei et al. (2014) employed a sophisticated combination of neuromechanical and pharmacological approaches to explore the role of 5-HT in spinal gain control. The experimental design involved participants performing a precision force task with the finger (upper limb) to assess motor output stability, while simultaneously performing an isometric contraction with the lower limbs at varying intensities. The rationale was that the remote contraction would diffusely increase the descending serotonergic drive to the spinal cord, thereby modulating motoneuron excitability. This hypothesis was further tested pharmacologically by administering 5-HT blockers and selective serotonin reuptake inhibitors (SSRIs) to manipulate 5-HT activity systematically. The central premise of the study was that an increased serotonergic drive would activate PICs in the motoneurons. PICs are known to amplify both the task-relevant (voluntary) and task-irrelevant (noise or fluctuation) synaptic input to the motoneuron, effectively increasing the motoneuron's input-output gain. This amplification was hypothesised to promote greater force-generating capacity but at the expense of force precision (i.e., increased force variability). Consistent with this model, the researchers observed that as the intensity of the remote lower-limb contraction increased, the force variability during the upper-limb precision task also increased, suggesting a dose-dependent effect of the remote contraction on spinal

excitability. Crucially, the pharmacological manipulations supported this interpretation: the administration of 5-HT blockers decreased force variability, while the SSRIs amplified force variability. This study provided robust evidence that the diffuse serotonergic projections, previously well-documented in animal models, might similarly function in humans to modulate motoneuron gain. Other robust techniques have been developed to estimate the diffuse 5-HT action.

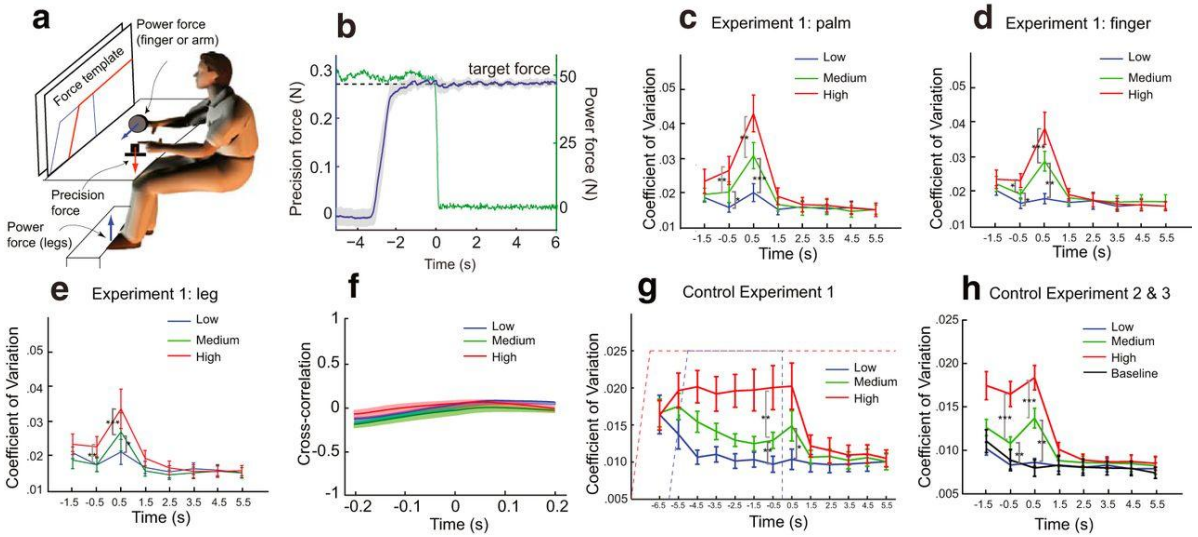

Figure 6. Effect of Serotonin on Motor Gain. Adapted with permission from Wei et al. (2014), licensed under CC-BY.

The lines on the screen represent the force instructions for the precision force (red) and the power force (blue). b, Force signals from a typical subject during the medium power force task. Subjects were instructed to produce a precision force with their left index finger (mean shown in blue) while first holding and afterward removing the power force. The green trace depicts a typical power force recording, dropping to half of its amplitude at time 0. Gray shading denotes SDs across trials. c–e, Variance (CV) of the precision force during each second before and after switching off the power force produced by the palm (c), the finger (d), and the leg (e). The moment when the power force drops to its half amplitude is defined as time 0. On the time axis, 0.5 s means that the variance is calculated over the first second after time 0 (between 0 and 1 s). Significant differences have been found between power force intensity levels within the −1, 1, and 2 s (not marked in graph) for all effectors. f, Cross-correlation of the power force and the precision force is plotted as a function of time lag for three force levels separately. g, Results from Control Experiment A, presented in the same format as in c. The dashed lines represent the force instructions for the precision force (red) and the power force (blue), which are now in the reverse order as a. Significant differences have been found between power force intensity levels within the −1, 1, and 2 s (not marked in graph). There is no difference found within −7 s. h, Results from Control Experiments B and C, presented in the same format as in c. Significant differences have been found between power force intensity levels within the −1, 1, and 2 s (not marked in graph). *p < 0.05, **p < 0.01, ***p < 0.001.

MUs decomposition and data analysis when using HDsEMG

High-density superficial electromyography (HDsEMG) is a non-invasive technique that records motor units (MUs) activity using a grid of closely spaced surface electrodes, typically through tens to hundreds of channels. HDsEMG can detect MU action potentials, which exhibit a unique spatial “fingerprint” across the electrode array. These fingerprints allow the recorded EMG signals to be decomposed into the activity of individual motoneurons. A widely used decomposition method is the Convolution Kernel Compensation (CKC). This blind-source separation technique extracts the MU firing train without prior knowledge, for example, about the number of MUs recorded or their exact discharge patterns (Holobar & Zazula, 2007). CKC mathematically compensates for the convolution process that occurs when the firing activity of multiple MUs overlaps in time and space within the recorded EMG. In summary, it detects the unique patterns of MU action potentials and separates their spike trains from the brute EMG signal, composed of other MUs’ signals, noise, and artifacts.

To illustrate, imagine listening to a choir accompanied by an orchestra. Within this scenario, instead of hearing the collective sound, you wish to isolate each singer’s voice. CKC can be used as an algorithmic “ear” that distinguishes and separates each singer’s unique vocal pattern, allowing us to analyse them individually. Because EMG recordings are noisy and MU action potentials often overlap, CKC relies on a statistical index based on the Mahalanobis distance, which indicates how closely a detected firing matches the expected MU pattern, thereby improving the accuracy of discharge identification (Zhang & Huang, 2015). CKC also permits tracking the same motor units over time, promoting longitudinal analysis of MU behaviour across conditions, allowing comparison of before-and-after interventions, or the influence of disease progression on motoneuron behaviour (Maathuis et al., 2008; Martinez-Valdes et al., 2017). Despite being highly automated, expert visual inspection and manual editing remain an important part of the decomposition process, enhancing its reliability and preventing the inclusion of unreal firings or artifacts (Machado et al., 2021; Zhao et al., 2024). Overall, HDsEMG combined with CKC decomposition represents a considerable advancement in the study of motoneuron physiology, allowing researchers to monitor the activity of multiple individual MUs non-invasively and track them across time.

Paired motor unit technique and ΔF

The paired motor unit (PMU) technique was initially proposed by Monica Gorassini et al. (2002; 1998) and is the current gold standard non-invasive method for indirectly estimating the magnitude of PICs in human motoneurons, validated in simulation, human, and animal studies (Powers & Heckman, 2015; Stephenson & Maluf, 2011; Vandenberk & Kalmar, 2014). This technique estimates the recruitment-de-recruitment hysteresis of motor units using HDsEMG data acquired during slow, ramp-up, and ramp-down isometric contractions at low to moderate force levels (e.g., 20-50% of maximum voluntary contraction) (Hassan et al., 2020; Stephenson & Maluf, 2011). A critical step is motoneuron decomposition, in which advanced algorithms are applied to HDsEMG signals to accurately identify the discharge patterns of numerous (usually tens) individual MUs (De Luca & Hostage, 2010). Following decomposition, MUs are paired following strict criteria. One or more lower-threshold MU (control units) that were recruited earlier are paired with a higher-threshold MU (test unit) that was recruited later. Control units must be recruited at least one second before the test unit, increasing the probability that PICs were fully activated, and must be active throughout the entire period of test unit activity, serving as a reliable measure of the common synaptic input to the motoneuron pool (M. Gorassini et al., 2002). The core of the technique is the calculation of delta F (ΔF), which quantifies the difference in firing frequency of the control unit at the moment the test unit is de-recruited versus the moment it was recruited (Afsharipour et al., 2020). Since the firing rate of the control unit is a proxy for the net synaptic input to the motoneuron pool, a positive ΔF value indicates that the test unit requires a lower net synaptic input to remain active (de-recruitment) than it did to be initially activated (recruitment). This difference reflects the self-sustained depolarisation provided by the PICs (Hassan et al., 2020). The magnitude of ΔF is thus an indirect measure of the PIC contribution to motoneuron excitability.

Known factors that may (or may not) influence PIC estimates

A growing body of literature has identified potential confounding factors (e.g., sex, age, and physical activity) that should be considered when investigating PICs. In contrast, the relevance of caffeine consumption, once considered a critical factor, has recently been questioned. For example, larger ΔF scores have been reported in female compared with male participants (Jenz et al., 2023), as well as in young

compared with older male participants (Orssatto et al., 2022, 2023). In addition, older participants have demonstrated impairments in the facilitation-inhibition control of motoneurons (Orssatto et al., 2022). Notably, the training level seemed important to partially mitigate these age-related differences, as increased ΔF was observed in older male participants after a 6-week resistance training program (Orssatto et al., 2023). Collectively, these findings suggest that motoneuron biophysical properties, neuromodulatory drive and inhibitory input across sex, age and training levels. Although it remains unclear whether these factors directly influence PIC responses, they warrant careful consideration in studies assessing PIC estimates.

In contrast, the influence of caffeine on PIC estimates remains conflicting. While research groups have controlled caffeine intake when investigating PICs (Gomes et al., 2024; Goreau et al., 2025), controversial evidence has been reported regarding its effects on motoneuronal gain. Caffeine acts as a competitor for adenosine receptors and can positively influence the monoaminergic drive (Grant & Eugene Redmond, 1982; Volkow et al., 2015), a mechanism that could theoretically increase PIC activity. Supporting this notion, previous studies have shown increased H-reflex recruitment curve slope and self-sustained motor unit firing frequency (Walton et al., 2002), higher motoneuron firing frequency (Phillis et al., 1979), and altered MU recruitment behaviour (Black et al., 2015) following caffeine consumption. However, Kirk et al. (2019) demonstrated that caffeine neither increased PIC estimates, assessed using neuromuscular electrical stimulation with tendon vibration (VIB+STIM), nor corticospinal excitability, investigated by transcranial magnetic stimulation (TMS). More recently, Mackay et al. (2023) similarly reported no effects of caffeine on ΔF and peak MU firing frequencies.

In summary, existing evidence indicates that motoneuron biophysical properties, neuromodulatory drive, and inhibitory input may differ between males and females, younger and older individuals, and trained versus untrained populations, and these factors should be carefully controlled or acknowledged when interpreting PIC estimates. Conversely, current findings suggest that controlling caffeine consumption may be less critical in studies of PICs than previously assumed.

Statistical analysis when using HDsEMG

Just as important as correctly treating data acquired with HDsEMG is using the appropriate statistical analysis. Since we can decompose several MUs for each participant, HDsEMG datasets will provide multiple observations nested within participants. This arrangement violates the premise of traditional statistical approaches [e.g., analysis of variance (ANOVA)] that all data points are independent. As a consequence, it would lead to an underestimation of within-subject variability and an inflation of Type I error (Yu et al., 2022). Alternatively, averaging all MUs per participant before analysis would discard valuable within-subject information, reducing the sensitivity to detect meaningful effects. This way, Linear Mixed Models (LMMs) become a more appropriate approach, including both fixed effects, which represent the primary variables of interest (e.g., experimental condition, time, or group), and random effects, representing the inherent variability between subjects (e.g., individual differences in MU behaviour). This promotes the inclusion of all decomposed MUs for each participant, while properly weighting them according to their participant-specific variability, thereby increasing statistical power and decreasing the chance of Type I error (De Melo et al., 2022; Yu et al., 2022).

A further refinement of this approach is the use of robust linear mixed-effects models (rLMMs), which are more resilient to violations of model assumptions and irregular data structures. In HDsEMG datasets, it is common to have missing or unbalanced data, for instance, when specific motor units cannot be decomposed for a given participant or condition. While ANOVA cannot handle such cases, standard LMMs may be affected by deviations from normality or heteroscedasticity. In contrast, rLMMs address these limitations through bounded-influence estimation, thereby reducing the impact on both fixed and random effects (Koller, 2016). In addition, rLMMs also offers the possibility of applying bootstrapping. This resampling-based method estimates confidence intervals empirically by repeatedly drawing samples from the data (Field & Welsh, 2007), reducing dependence on theoretical distributional assumptions and improving the reliability of parameter estimates. Thus, robust LMMs propose to increase the reliability of estimates obtained through HDsEMG, even in the presence of an unbalanced or incomplete HDsEMG dataset.

ΔF as an estimate of motoneuron excitability after a remote contraction

Using the PMU technique, Orssatto et al. (2022) and Mackay Phillips et al. (2023) investigated the effects of remote contraction on PICs in lower limb motoneurons. Both studies employed a remote voluntary submaximal isometric handgrip contraction to diffusely increase the descending serotonergic drive to the spinal cord, with the PIC contribution estimated by calculating ΔF from the soleus and/or tibialis anterior motor unit discharge patterns during slow, ramp contractions. Orssatto et al. (2022) estimated PICs in young and older adults, finding that the handgrip successfully facilitated PICs (increased ΔF) in both soleus and tibialis anterior motoneurons. Importantly, this facilitatory effect was diminished but preserved in older adults (Figure 7). Mackay Phillips et al. (2023) found that the remote handgrip contraction increased ΔF in the soleus motoneurons. These studies provide evidence, via the PMU technique, that a remote contraction in the upper limb is an efficient non-pharmacological method for investigating PICs estimates in lower limb motoneurons, supporting the hypothesis of diffuse, cross-limb serotonergic facilitation of motoneuron excitability in humans.

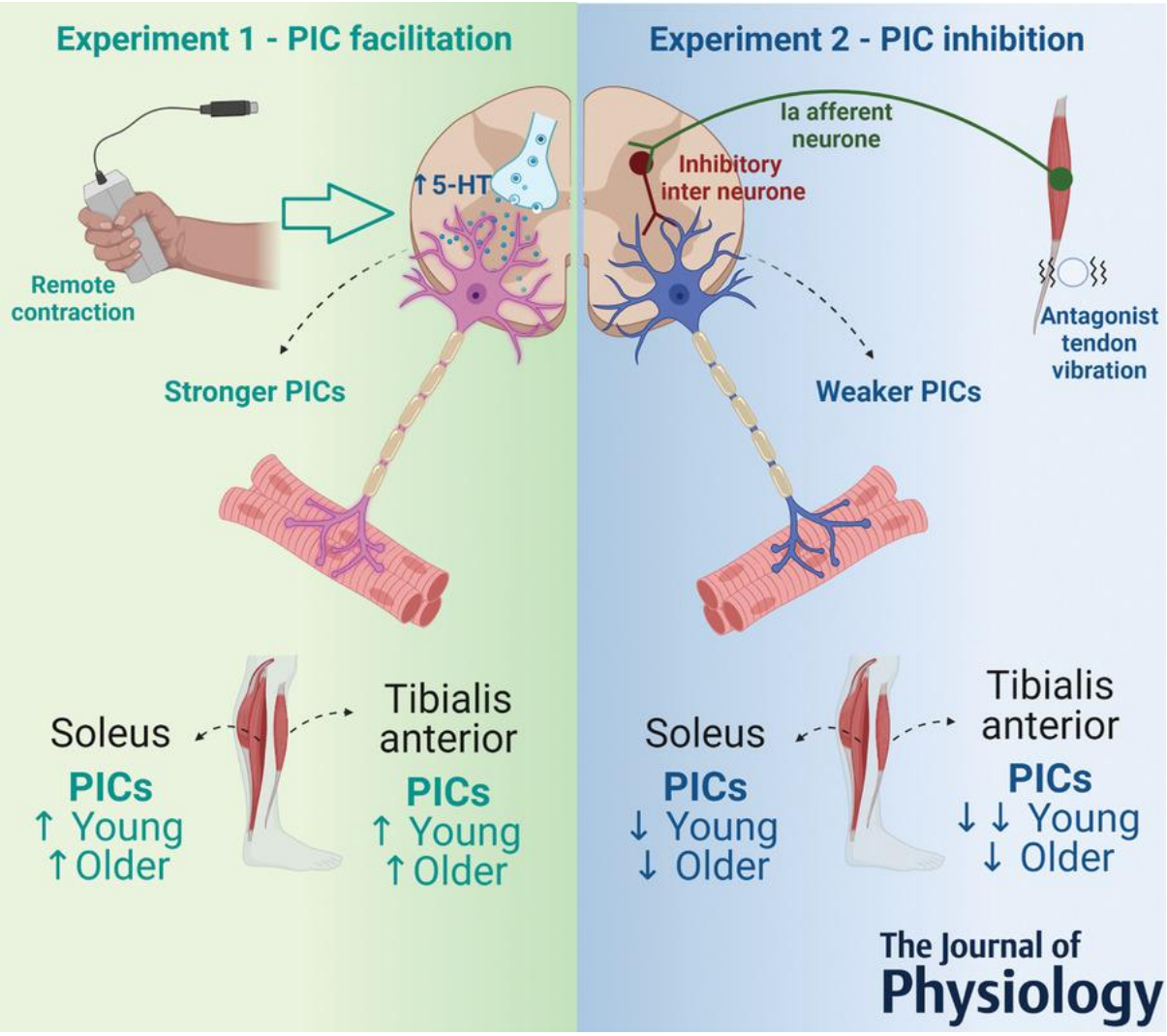

Figure 7. Representation of persistent inward currents facilitation in lower limbs motoneurons through a remote handgrip contraction.

Both young and older adults showed increased excitability in the soleus and tibialis anterior motoneurons through estimates of PICs (ΔF), though a smaller response was observed in the older. Reproduced from Orssatto et al. (2022) in The Journal of Physiology under the Creative Commons Attribution (CC BY) 4.0 International License.

While previous studies have provided compelling evidence for the diffuse nature of the serotonergic system and its role in modulating motoneuron excitability in humans, a critical gap remains in understanding variables that govern this neuromodulatory mechanism. Although remote voluntary contraction is an effective non-pharmacological method for, theoretically, increasing spinal 5-HT drive and, consequently, PICs estimates, existing evidence has primarily focused on the effects of contraction intensity. Importantly, increases in contraction intensity are inherently accompanied by increases in the area under the force/torque-time curve (e.g., the time-torque integral, or "impulse"), and similar increases in impulse can also be achieved by prolonging contraction duration. As a result, it remains unclear whether

the magnitude of the neuromodulatory effect on motoneurons not directly involved in the task is primarily driven by contraction intensity, contraction duration, or the resulting contraction impulse. The study presented in this doctoral thesis is designed to address this fundamental research gap by clarifying how the mechanical variables of a remote contraction can be manipulated to control the magnitude of 5-HT-mediated neuromodulation. The expected findings hold practical relevance, since this information can be used by athletes and clinicians to optimise motor performance and rehabilitation strategies; and fundamentally, our results will contribute to a deeper understanding of this critical spinal mechanism, which is essential for developing targeted pharmacological interventions for neurodegenerative diseases and advancing the precision of brain-machine interface technologies.

Novel approaches to estimate neuromodulatory and inhibitory inputs

Although ΔF has been established as a valuable proxy for estimating PIC contribution to motoneuron excitability, it is not without limitations. Because ΔF is influenced by both the magnitude of neuromodulatory input and the specific pattern of synaptic inhibition, added to the fact that quantifying the monoaminergic activity in humans is virtually impossible, interpretation of the mechanism influencing changes in ΔF remains inherently constrained. Consequently, distinguishing whether alterations in ΔF arise predominantly from changes in neuromodulatory drive or inhibitory control is challenging. To address this limitation, Beauchamp et al. (2023) introduced a reverse-engineering approach to analyse non-linearities in motor unit discharge behaviour. Using biophysical simulations of over 300,000 combinations of excitatory, inhibitory, and neuromodulatory inputs, the authors demonstrated that specific geometric features of the discharge-torque relationship are sensitive to the underlying neural drive. In other words, this approach is an attempt to dissociate the metabotropic (monoaminergic) influences from ionotropic (inhibitory) mechanisms during voluntary contractions, thereby providing a novel non-invasive approach for investigating PIC-related effects in humans.

The primary metric proposed is the so-called "brace height," defined as the maximum orthogonal deviation of the smoothed motor unit discharge trajectory from a linear line connecting recruitment to peak firing. This value is typically normalised to a theoretical right triangle, representing the maximum possible "bowing" of the curve

if the motoneuron were to reach firing rate saturation immediately upon recruitment. From a physiological perspective, increased descending release of 5-HT and NE within the spinal cord is expected to enhance PIC activation, resulting in a steeper, non-linear acceleration of discharge rates immediately after recruitment (i.e., within the secondary firing range). Within this framework, Beauchamp et al. (2023) demonstrated that brace height is highly sensitive to the magnitude of neuromodulatory input. Moreover, whereas ΔF primarily reflects PIC-induced prolongation of discharge (hysteresis), brace height captures the amplification of synaptic input, thereby offering a complementary and broader perspective on the neuromodulatory mechanisms (Mesquita et al., 2024).

In contrast, the "attenuation slope" was proposed as a metric associated with the pattern of inhibitory input, such as proportional or reciprocal inhibition. Attenuation slope represents the rate of change in discharge rate during the tertiary range and is calculated between the geometric inflection point and peak firing. The rationale is that proportional, or "balanced", inhibition linearises the discharge trajectory, thereby substantially reducing the attenuation slope. Importantly, attenuation slope is mathematically dependent on brace height, as the point of maximum orthogonal deviation defines the onset of the attenuation segment. Consequently, changes in attenuation slope must be interpreted cautiously, as they may reflect alterations in inhibitory input, changes in brace height, or an interaction between both mechanisms.

Study

Introduction

Motoneurons rely on a complex control system for excitability, with neuromodulation as a key component. Unlike ionotropic systems, which directly convert synaptic inputs into action potentials by opening ion channels, neuromodulation operates via neurotransmitters that bind to specific receptors, initiating intracellular signalling pathways that regulate motoneuron responsiveness (Heckman et al., 2009). This neuromodulatory influence facilitates the generation of strong PICs in spinal motoneurons, thereby increasing cell excitability and accelerating, amplifying, and prolonging their discharge in response to a given excitatory input (Heckman, Johnson, et al., 2008). This input–output gain mechanism can be adjusted based on 5-HT and NE released from the raphe nuclei and locus coeruleus, which project to the spinal cord (Heckman, Hyngstrom, et al., 2008) and activate receptors on motoneuron dendrites. This activation promotes an intracellular response via second messengers, leading to the opening of L-type Ca^{2+} and Na^{+} channels and facilitating PICs in a dose-dependent manner (Johnson & Heckman, 2014). It has been suggested that the level of neuromodulatory input onto the motoneurons could be adjusted according to the physical tasks' demand (Heckman et al., 2009). Lower levels of monoaminergic drive would lack the facilitation in recruited motoneurons, minimising involuntary synaptic noise and allowing precise movements (e.g., threading a needle). Alternatively, moderate-to-maximal motor tasks (e.g., lifting heavy weights) require a higher monoaminergic drive and an increased state of PIC facilitation (Johnson & Heckman, 2014). This theory is supported by the observation that the firing rate of neurons in the caudal raphe nuclei is influenced by motor output intensity, such that 5-HT release is proportional to locomotion demands (Fenstermacher et al., 2024; Veasey et al., 1995). Importantly, most current knowledge of neuromodulation effects on PICs and 5-HT’s role in motoneuron gain control comes from invasive animal experiments and computational simulations (Heckman et al., 2009; Hounsgaard et al., 1988; Lee & Heckman, 2000). However, translating these findings to humans remains challenging and requires non-invasive techniques.

In humans, researchers have used different strategies to investigate the contribution of 5-HT and NE to PIC neuromodulation through indirect measures. Pharmacological trials involving drugs that alter the concentration of 5-HT and NE in the synaptic cleft have demonstrated their influence on PIC estimates and indicators of motoneuron excitability (e.g., discharge rate, recruitment threshold) (D'Amico et al., 2013; Goodlich et al., 2023, 2024; Udina et al., 2010). Additionally, some studies have utilised muscle contractions of varying intensities to theoretically enhance monoaminergic input to the spinal cord, aiming to understand its effects on PICs. Although some studies observed no increase (Afsharipour et al., 2020; Kim et al., 2020), others report that PIC contribution to motoneuron firing increases with contraction intensity within the same muscle group (Mackay et al., 2023; Orssatto et al., 2021), while the effect appears greater at higher force levels (Škarabot et al., 2025). The experimental strategy of using remote contractions to modulate motoneuron excitability is supported by the work of Wei et al. (2014), who showed that remote leg contractions increased force variance during a precision task with the palm or index finger. This effect was interpreted as 5-HT-mediated, as force variation decreased in the presence of selective 5-HT receptor blockers and reuptake inhibitors. Building on this evidence, other studies have since employed remote handgrip contractions to theoretically increase 5-HT availability and thereby amplify PIC estimates in lower-limb muscles (Mackay Phillips et al., 2023; Orssatto et al., 2022). These findings align with evidence that the firing rate of neurons in the caudal raphe nuclei and locus coeruleus is influenced, respectively, by motor outflow intensity and arousal, such that 5-HT and NE release is proportional to task or locomotion demands (Fenstermacher et al., 2024; Heckman, Johnson, et al., 2008; Veasey et al., 1995). Collectively, these findings align with animal evidence and support the feasibility of indirectly investigating serotonin's contribution to motoneuron firing modulation through remote muscle contractions.

After establishing the efficacy of remote handgrip tasks in increasing 5-HT input onto the spinal cord and facilitating tibialis anterior and soleus PICs (Mackay Phillips et al., 2023; Orssatto et al., 2022), the next step was to understand whether PIC responses are differently influenced by mechanical aspects of remote contractions (i.e., force intensity, duration, and impulse). This rationale is supported by evidence that higher contraction intensities increase estimates of PIC contribution to self-

sustained motoneuron firing (e.g., ΔF) in the soleus, gastrocnemius (Orssatto et al., 2021) and tibialis anterior (Mackay et al., 2023), suggesting that intensity modulates PIC activation. Additionally, serotonergic neuron activity in the raphe nuclei of cats rises and maintains during prolonged locomotion until the cat no longer maintains pace (Jacobs et al., 2002), implying that sustained motor activity may also enhance PIC functional contribution (i.e., increase their estimated magnitude or effect on motoneuron excitability). To investigate this phenomenon, the present study used multi-channel electromyography to assess MU discharge rates, and the paired-MU technique to estimate PICs' contribution to motoneuron firing (M. A. Gorassini et al., 1998; Mesquita et al., 2024) following handgrip contractions at different intensity levels and contraction durations. In addition, we estimated the non-linearity of motoneuron discharge patterns caused by monoaminergic drive using a quasi-geometric approach (‘brace height’) applied to the ascending phase of the MU firing rates (Beauchamp et al., 2023). Given the evidence that contraction intensity affects PIC estimates in motoneurons (Mackay et al., 2023; Orssatto et al., 2021) and higher motor output potentially elevates 5-HT concentration in the spinal cord and potentially increases motoneuron excitability (Jacobs et al., 2002), we hypothesize that the combination of both greater remote contraction intensity and duration (i.e., impulse) will induce higher PIC estimates.

Methods

Ethical approval

All procedures conformed to the ethical standards set by the latest revision of the Declaration of Helsinki, except for registration in a public database. All participants provided written informed consent prior to participation. The study was approved by the Queensland University of Technology Human Research Ethics Committee (Reference number: 6770).

Participants and ethical procedures

Twenty-three young adults participated in this study (Table 1). Inclusion criteria required volunteers to be within the age range of 18–40 years, have no history of musculoskeletal injuries in the tested limbs, and not be using medications that could

affect the monoaminergic system (e.g., beta-blockers and selective serotonin reuptake inhibitors) (Thorstensen et al., 2024). Furthermore, participants were instructed to abstain from strenuous physical activities and alcohol consumption for 48 hours before the testing session.

Study design and testing procedures

The dorsiflexion and handgrip contractions were performed using the “preferred leg for kicking a ball” and the corresponding ipsilateral hand, respectively. For the dorsiflexion tasks, participants were positioned upright in an isokinetic dynamometer (Biodex System 4, Biodex Medical System, Shirley, NY) with the knee fully extended (0˚), seat reclined at 70˚ of hip flexion and ankle in anatomical position (Figure 8). For the handgrip tasks, a handgrip dynamometer (model MLT004/ST, ADinstruments, Australia) was held with the shoulder in the anatomic position at 0° flexion/extension, 0° abduction/adduction, and 10-15° external rotation, with the elbow flexed at 90˚, while seated on the isokinetic dynamometer. A warm-up was performed alternating between handgrip and ankle dorsiflexion tasks, and consisted of progressive contraction levels (20%, 40%, 60%, 80% of perceived maximal effort) for ~5 s each. Two minutes later, they performed two 4-s handgrip maximal voluntary contractions, with 60 s of interval between attempts. They were given two minutes of rest and then also performed two 4-s dorsiflexor maximal voluntary contractions, with 60 s of interval between attempts. Peak handgrip force and dorsiflexion torque were considered as the maximum value achieved during the maximal voluntary contractions. Thereafter, participants were familiarised with triangular-shaped ramped contractions to 20% of their dorsiflexion peak torque at a 2%/s rate of torque increase and decrease (10-s up and 10-s down). Visual feedback was standardised for all participants, provided through a 23” computer monitor, y-axis range from 0 to 22% of peak torque, position standardised at ~150 cm from the participant, and the software window maximised. Participants were guided to closely follow the real-time torque trajectory by keeping a small yellow ball close to the requested torque trajectory. They received the following explicit verbal instructions: “Gradually increase and decrease your force, using your whole foot–not just your toes–to keep the yellow ball on the triangular line, avoiding abrupt increases or decreases of force generation". This relative torque level was chosen based on previous studies investigating the effects of a remote handgrip contraction on lower limbs ΔF (Mackay Phillips et al., 2023; Orssatto et al., 2022).

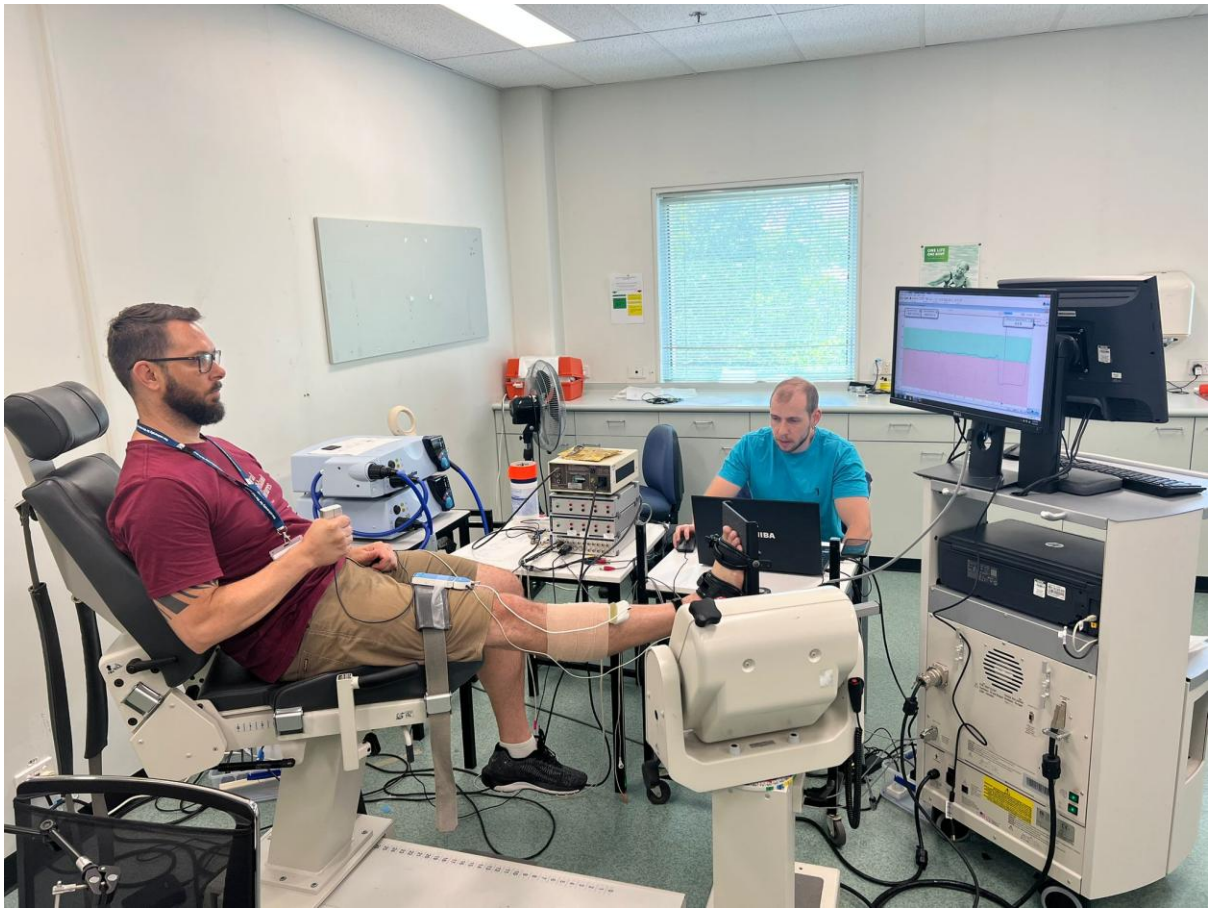

Figure 8. Experimental setup for the ankle dorsiflexion and handgrip tasks.
Participant positioned in the isokinetic dynamometer for ankle dorsiflexion torque assessment, while simultaneously performing ipsilateral handgrip contractions. Visual feedback of the target and real-time torque trajectory was provided on a computer monitor.

Five minutes after familiarisation, four clusters of two sets of triangular-shaped contractions, interspersed with either a resting control condition or three distinct handgrip contractions, were performed in a randomised order. During the control condition, participants were asked to remain quiet and relaxed, avoiding moving/contracting any muscle for 60 s in between the two triangular-shaped contractions. For the three conditions involving a remote handgrip contraction, participants were requested to quickly reach a relative grip force level and sustain it for a given time; the three conditions were i) handgrip contraction at 40% of their peak force sustained for 15 s (i.e., 40%15s); ii) handgrip contraction at 40% of their peak force sustained for 30 s (i.e., 40%30s), and iii) handgrip contraction at 80% of their peak force sustained for 15 s (i.e., 80%15s). Conditions 80%15s and 40%30s were impulse matched, 80%15s and 40%15s were time-matched, and 40%30s and 40%15s

were intensity matched. This approach was adopted to allow us to investigate whether the increases in ΔF are affected by remote contraction time (40% sustained for 15 s vs 30 s), intensity (40% vs 80% sustained for 15 s), or impulse (40% sustained for 30 s and 80% sustained for 15 s vs 40% sustained for 15 s). The time between the two triangular-shaped contractions, performed before and after the control or handgrip conditions, was standardised at 60 s. Thus, a 30-s waiting period preceded the handgrip contraction lasting 30 s, while a 45-s waiting period preceded the handgrip contraction lasting 15 s. The second triangular-shaped contractions were performed immediately after the handgrip conditions. A 5-min rest interval was adopted between conditions. Figure 1 illustrates the study protocol design. In case abrupt/steep increases or decreases (>5% of peak torque) of torque were observed during the ascending or descending phase of the triangular-shaped contractions, the whole trial for the specific condition was excluded and repeated after 5 minutes. The participants were given only one extra attempt for each condition when necessary. No further attempts were given to avoid the presence of fatigue, which can affect the outcomes obtained from the triangular contractions (Mackay et al., 2023). If the participant was not able to maintain consistency in the before and after triangular ramp-shaped contractions (i.e., avoid abrupt/steep increases or decreases in torque) for both attempts in a given condition, they were excluded from the analysis for that condition. Finally, the impulse (area under the force curve) was calculated at the handgrip conditions using the trapezoidal rule in LabChart software (version 7.3, ADInstruments, Australia).

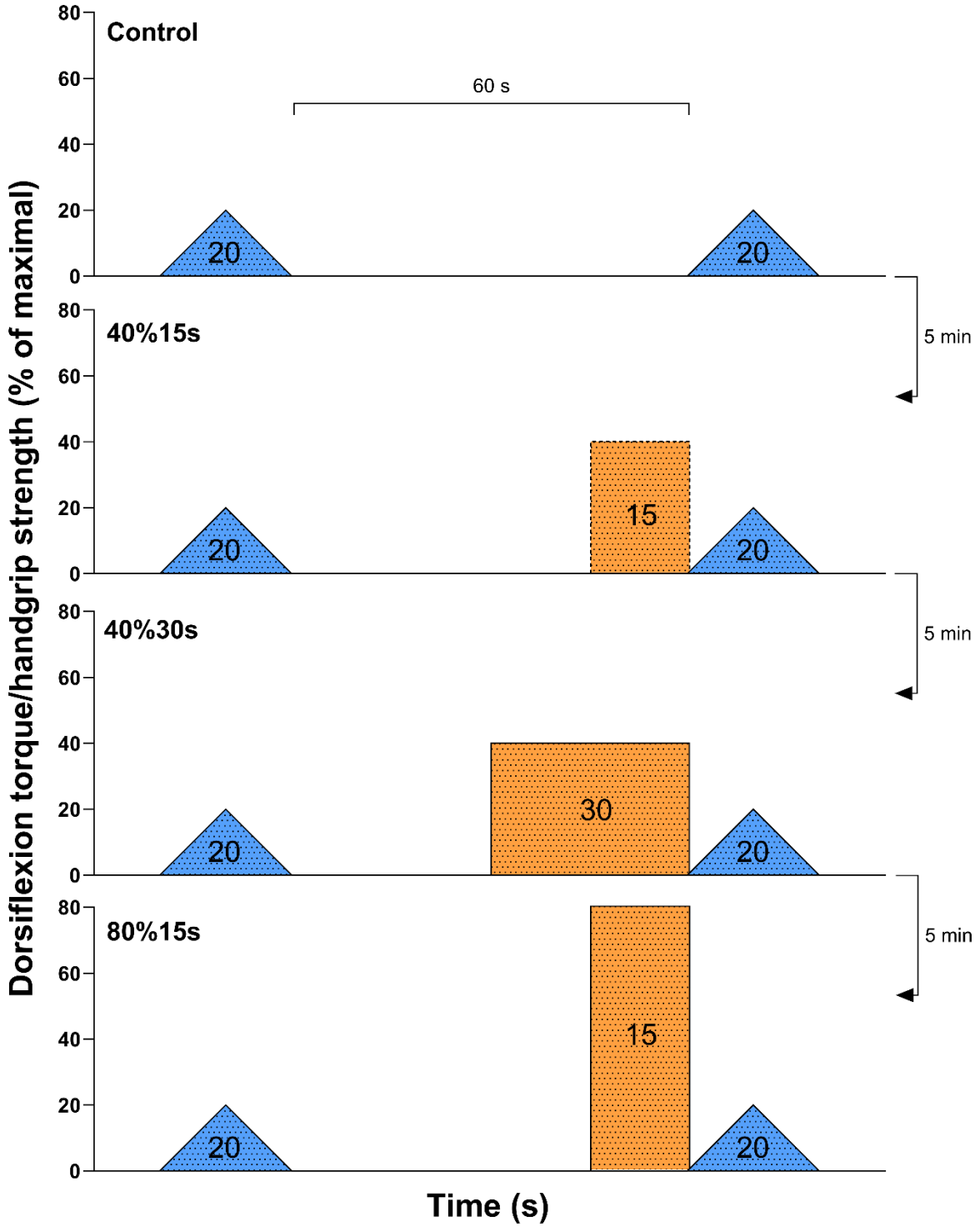

Figure 9. Study protocol.

Blue triangles represent the triangular ramp-shaped dorsiflexion contraction with the time between these two tasks set at 60 s for all conditions; the dashed orange rectangle represents half handgrip impulse as the other two solid-lined orange rectangles; conditions were performed randomly with 5 min rest interval.

Multi-channel Electromyography Recordings and Analyses

The tibialis anterior skin area was prepared by shaving and cleansing with 70% isopropyl alcohol. A semi-disposable 64-channel electrode grid with an interelectrode distance of 8 mm (GR08MM1305, OT Bioelettronica, Turin, Italy), attached to a bi-adhesive foam layer and coated with a conductive paste (Ten20, Weaver and Company, Aurora, CO, USA), was positioned over the most prominent part of the tibialis anterior. The grounding electrode comprised a dampened strap (WS2, OTBioelettronica, Torino, Italy) placed around the ankle joint. Multi-channel electromyograms were recorded during the triangular ramp-shaped contractions using monopolar mode, amplified (256×), subjected to band-pass filtering (10–500 Hz), and digitally sampled at 2048 Hz, with a 16-bit wireless amplifier (Sessantaquattro, OT Bioelettronica, Turin, Italy), interfacing with OTBioLab + software (version 1.3.0., OT Bioelettronica, Turin, Italy) and were stored for subsequent offline analysis. The recorded data was processed offline using the DEMUSE software (Holobar & Zazula, 2007). The signals were band-pass filtered (20–500 Hz) through a second-order, zero-lag Butterworth filter. Subsequently, MU decomposition was performed using the convolutive kernel compensation (CKC) method (Holobar et al., 2014; Holobar & Zazula, 2007). Following decomposition, the same MUs were tracked across the before and after handgrip or control conditions, but not across conditions. The files for each contraction were concatenated, and separation vectors (i.e., MU filters) were used to identify and generate the MU spike trains across the two contractions (Del Vecchio et al., 2019; Holobar et al., 2014; Holobar & Zazula, 2007). Then, a trained investigator (LU) examined the MU spike trains and, when necessary, edited the discharge patterns of the MUs (Del Vecchio et al., 2020). Only MUs with a pulse-to-noise ratio ≥ 30 dB were retained for presenting higher reliability (sensitivity > 90% and false alarm rates < 2%) (Holobar et al., 2014). The edited MUs were quality-checked by a researcher, blinded for each condition, (LBRO) with extensive experience in decomposing, tracking and editing of MUs. When no MU was identified in both contractions for a given condition, the participant was not included in the respective condition analysis.

Estimation of PIC contribution to motoneuron self-sustained firing (ΔF) and peak discharge rate

The torque signal has been filtered using a 5th-order, low-pass Butterworth filter with a cut-off frequency of 10 Hz. Then, the MU discharge events were converted into instantaneous discharge rates and smoothed with a support vector regression machine learning fit (Beauchamp et al., 2022). PIC contribution to motoneuron firing was estimated using the paired MU analysis (Afsharipour et al., 2020). MUs with lower recruitment thresholds (control units) were paired with units of higher recruitment threshold (test units). ΔF was calculated as the change in discharge rates of the control MU from the onset of recruitment to the point of de-recruitment of the test unit (Afsharipour et al., 2020). MUs were paired when the following criteria were obtained between control and test units i) a rate-to-rate correlation threshold of $r \geq 0.7$, ii) test – control unit recruitment time >1 s, iii) the control unit's discharge rate at test unit recruitment minus the control unit's peak discharge rate was > 0.5 pps, iv) control unit derecruitment time > test unit derecruitment time (Afsharipour et al., 2020; Hassan et al., 2020; Vandenberk & Kalmar, 2014). ΔFs calculated for each individual test unit were averaged across control units to yield a singular ΔF value for each corresponding test MU. In the case that no MU pair was identified in both contractions for a given condition, the participant was not included in the analysis for that respective condition(s). The highest value derived from the support vector regression fit curve was determined as peak discharge rate. The relative torque (%) produced during the recruitment time of each MU was identified as MU recruitment threshold.

Estimation of neuromodulatory and inhibitory drive contributions to MUs firing changes

We used the quasi-geometric approach to estimate the neuromodulatory and inhibitory drive contributions to motoneuron firing, as proposed by Beauchamp et al. (2023). Firstly, we generated a straight line (hypothenuse) from the discharge rate at MU recruitment to the peak discharge rate of the support vector regression (SVR) smoothed curve. Then, we calculated the ‘brace height’, which represents the maximum deviation of the smoothed discharge rates trace from linearity (hypothenuse). The maximal orthogonal distance between the hypothenuse and the smoothed MU discharge trace was considered the brace height. Thereafter, brace height was normalised as a percentage of the maximal orthogonal distance between the straight line and a right triangle in which sides originate from the hypotenuse. We

also calculated the ‘attenuation slope’, which is proposed to be associated with the inhibitory input effect on MU discharge (Beauchamp et al., 2023). Attenuation slope was calculated from the brace height insertion on the smoothed discharge rates to the peak discharge rates of the ascending phase.

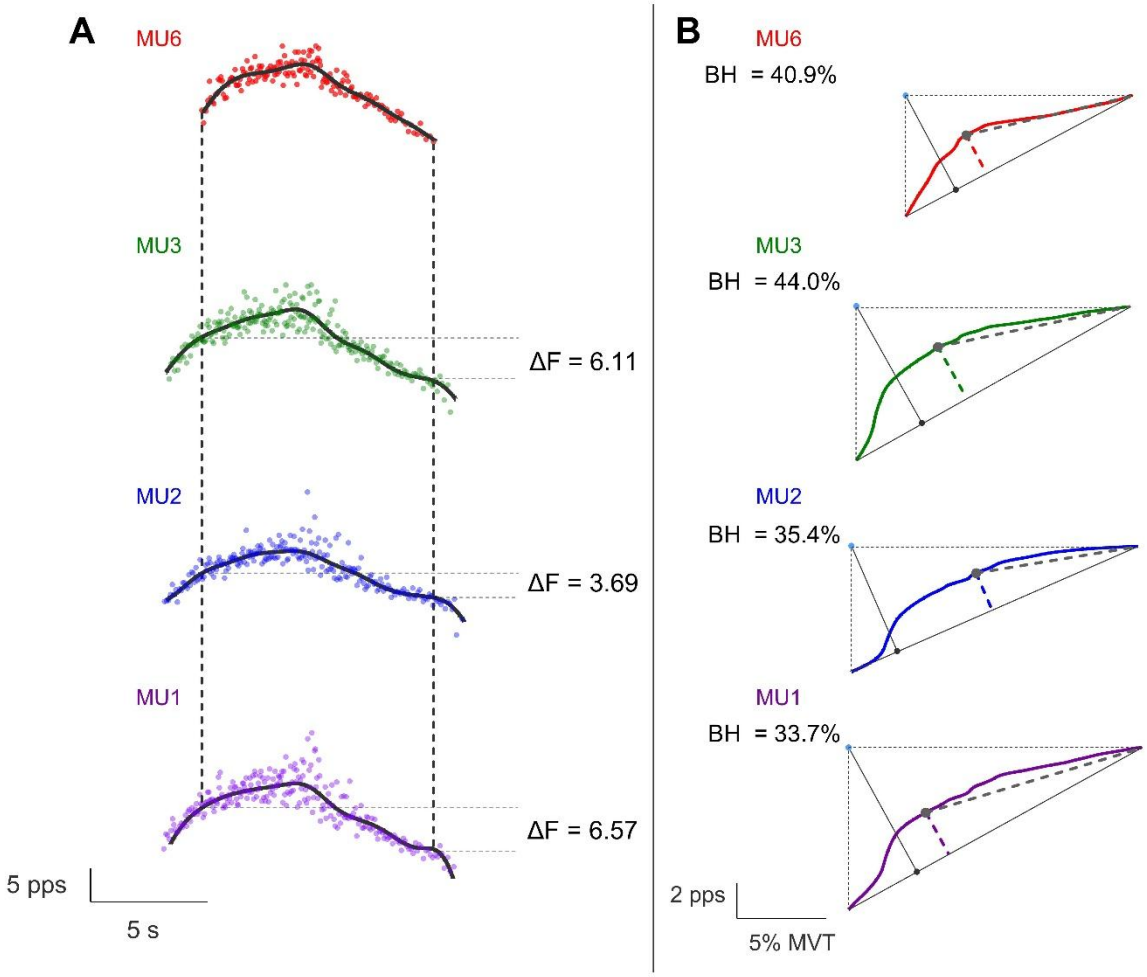

Figure 10A. Representation of ΔF calculation.

Red was used to represent firing of the test unit (top), paired with other three control units (green, blue and purple). Solid black lines, SVR smoothed curve of the MUs; dashed vertical black lines, moment of recruitment and decruitment of the test unit; dashed horizontal grey lines, firing of control units at the moment of recruitment and derecruitment of the test unit, used to calculate ΔF.

Figure 10B. Representation of brace height and attenuation slope calculation.

Solid coloured lines, SVR smoothed curve of the MUs; dashed coloured lines, brace height; solid black line, orthogonal distance originating from the hypotenuse (blue dot) to the right triangle (black dot) formed by the moment of recruitment to derecruitment of each MU (dashed black lines); dashed grey lines, attenuation slope; grey dots, point where brace height inserts and attenuation slope starts.

Data and statistical analyses

Linear-mixed effects models were investigated utilizing the *robustlmm* package (Kuznetsova et al., 2017). We estimated marginal mean differences between handgrip conditions in ΔF, peak discharge rate, brace height and attenuation slope along with 90% and 95% confidence intervals (CI), using the emmeans package (Lenth et al., 2023). Each MU was treated as repeated measure and nested for each participant, including a random intercept for each participant to consider for the correlation between repeated observations on each individual (i.e., 1| participant/MU ID). For ΔF, four distinct models were fitted: 1) time and condition were included as fixed effects and a random intercept and slope (ΔF) for each participant; 2) similar to model 1, but included peak discharge rate as covariate; 3) similar to model 1, but included recruitment threshold as covariate; 4) similar to model 1 but included both peak discharge and recruitment threshold as covariates. All the four models resulted in similar outcomes; thus we presented the findings derived from Model 4, which exhibited superior fit (as indicated by the lowest Akaike's information criteria and Bayesian information criteria). We performed an additional analysis by adding sex as fixed effect to ΔF to investigate a possible effect of sex on results, but no time by condition by sex interaction effect [β = 0.32 (-0.29, 0.92) pps, SE = 0.32; t = 0.31] and main effect of sex [β = -0.80 (-1.76, 0.16) pps, SE = 0.49; t = -1.64] were observed, so sex was removed from the model. For all other variables except by recruitment thresholds (i.e., peak discharge rate, brace height and attenuation slope), a model with time and condition as fixed effects and recruitment thresholds as covariate was employed. For recruitment thresholds, the model included time and condition as fixed effects. We also used the robustlmm package to check the consistency of the participants in performing the triangular-shaped contractions by comparing the area formed by the detrended torque trace between the requested and the performed triangular contractions. In addition, the mean impulse for each condition were compared using the *lmerTest* package for linear mixed effects models analysis. Pairwise *post hoc* analysis was used for significant effects observed. Cohen's d effect sizes were computed for the differences between after and before handgrip conditions, leveraging the population standard deviation (σ) estimated from corresponding robust linear mixed-effects models as the denominator (i.e, d = mean difference / σ). For interpretation, we considered Cohen's d <0.20 trivial; 0.20–0.49 small; 0.50–0.79

moderate; ≥0.80 large. A significance threshold of 5% (α level) was adhered to for all tests. All the analyses were conducted in a free software environment (RStudio, version 2024.12.1). The complete dataset and corresponding R script are accessible at https://github.com/lugliara/PICs.

Results

Participant characteristics and MU identification

One female participant was excluded from all the analyses due to inconsistencies in performing the ramp-shaped triangle contractions in all the conditions and another female participant was excluded because no MUs were identified at any condition. The final analysis included data from 21 participants. Among these 21 participants, all of them were included in the control and 80% of peak force for 15 s conditions (80%15s). For the 40% of peak force for 15 s (40%15s), data from five participants were excluded from the analyses because they were unable to consistently follow the force trace path during the triangular-shaped contractions without steep increases and decreases of force production (N = 3), or MUs did not meet essential prerequisites to form pairs (N = 2). For the 40% of peak force for 30 s (40%30s), data from three participants were excluded from the analyses because they were unable to follow the feedback path (i.e., detected abrupt/steep increases or decreases >5% of peak torque) during the triangular-shaped contractions (N = 2) or no MUs were detected (N = 1). In order to investigate the influence of excluding some trials on our results, we performed a sensitivity analysis including only the participants who successfully concluded all four conditions (N = 14). The main findings remained largely consistent with the original analysis. Although a Time by Condition interaction effect for Brace Height was no longer statistically significant, this does not affect the interpretation of ΔF, which is our primary outcome. The data and results for this analysis have been made publicly available on https://github.com/lugliara/PICs for transparency.

A total of 341, 249, 245 and 316 MUs were successfully decomposed and matched before and after the control (mean (95% CI) = 16.2 (13.9, 18.6), N = 21), 40%15s (15.6 (13.3, 17.8), N = 16), 40%30s (13.6 (10.8, 16.4), N = 18) and 80%15s (15.0 (12.2, 17.9), N = 21) conditions, respectively. This resulted in 199 (9.5 (7.8, 11.2),

N = 21), 146 (9.1 (7.2, 11.1), N = 16), 137 (7.6 (5.7, 9.6), N = 18) and 191 (9.1 (6.8, 11.4), N = 21) test units, respectively.

Handgrip contraction impulse

The impulse generated during the handgrip contractions during 40%30s (estimated marginal mean = 5639 (95%CI: 5054, 6224) N·s) and 80%15s (5667 (95%CI: 5082, 6252) N·s) were similar (p = 0.868), but superior than 40%15s (2933 (95%CI: 2348, 3519) N·s, p < 0.001).

Torque trace deviation from the requested path during the triangular contractions

No Time by Condition interaction effect was observed [β = -0.66 (-17.0, 3.69) % of peak torque·s, SE = 5.28; t = -1.26]. The estimated marginal means (95%CI) for the area formed before and after intervention were: Control [63.8 (56.6, 71.0) % of peak torque·s; 59.1 (51.9, 66.2) % of peak torque·s], 40%15s [65.7 (57.9, 73.5) % of peak torque·s; 58.9 (51.1, 66.7) % of peak torque·s], 40%30s [61.8 (54.3, 69.3) % of peak torque·s; 63.6 (56.1, 71.1) % of peak torque·s], and 80%15s [64.0 (56.9, 71.2) % of peak torque·s; 66.0 (58.8, 73.1) % of peak torque·s].

Regarding the area formed during the ascending and descending phases, no Time by Condition by Phase interaction effect was observed [β = 7.92 (-4.09, 19.9) %MVC, SE = 6.13; t = 1.29]. The estimated marginal means (95%CI) for the area (%MVC) formed, respectively before and after intervention, and for ascending and descending phases, were: Control [28.5 (23.9, 33.0); 27.6 (23.0, 32.1); 34.8 (30.3, 39.4); 30.3 (25.8, 34.9)], 4015 [29.2 (24.2, 34.3); 25.4 (20.3, 30.5); 33.1 (28.0, 38.2); 33.5 (28.5, 38.6)], 4030 [29.1 (24.3, 34.0); 28.2 (23.4, 33.0); 30.7 (25.8, 35.5); 34.0 (29.1, 38.8)], 8015 [27.0 (22.5, 31.6); 28.3 (23.8, 32.9); 37.4 (32.9, 42.0); 36.9 (32.4, 41.5)]. The data, scripts and results for this analysis have been made publicly available on https://github.com/lugliara/PICs.

Table 1. Participant characteristics

Participant characteristics	
Age (years)	31 (29, 33)

Sex (n)	
Male	15
Female	6
Body mass (kg)	79 (73, 86)
Height (cm)	177(173, 181)
BMI (kg/m^2)	25 (24, 27)
Handgrip peak force (N)	437 (386, 488)
Dorsiflexion peak torque (N·m)	54 (49, 60)

Participant characteristics data are presented as means with 95% confidence interval.

MU outcomes

ΔF

A time by condition interaction effect was observed β = -0.40 (-0.62, -0.18) pps, SE = 0.11; t = -3.60]. ΔF increased from before to after the intervention on 40%30s [0.33 (0.16, 0.49) pps, *d* = 0.47 (0.23, 0.72)] and 80%15s [0.24 (0.09, 0.38) pps, *d* = 0.34 (0.14, 0.55)], but remained unchanged on 40%15s [0.10 (−0.06, 0.26) pps, *d* = 0.15 (-0.09, 0.38)] and control [-0.07 (-0.21, 0.06) pps, *d* = -0.11 (-0.31, 0.09)] conditions (Figure 3).

Brace height

A time by condition interaction effect was observed [β = -2.56 (-5.06, -0.06) % rTri, SE = 1.27; t = -2.01]. Brace height increased from before to after the intervention on 40%30s [2.24 (0.18, 4.30) % rTri, *d* = 0.20 (0.02, 0.39)] and 80%15s [2.45 (0.64, 4.25) % rTri, *d* = 0.22 (0.06, 0.39)], but remained unchanged on 40%15s [1.86 (-0.15, 3.87) % rTri, *d* = 0.17 (-0.01, 0.35)] and control [-0.11 (-1.84, 1.61) % rTri, *d* = -0.01 (-0.17, 0.15)] conditions.

Attenuation slope

A time by condition interaction effect was observed [β = 0.06 (0.02, 0.10) pps/%MVT, SE = 0.02; t = 2.73]. Attenuation slope decreased from before to after the intervention on 40%30s [-0.05 (-0.08, -0.01) pps/%MVT, *d* = -0.27 (-0.46, -0.08)]and 80%15s [-0.03 (-0.06, -0.00) pps/%MVT, *d* = -0.17 (-0.34, -0.01)], but remained unchanged on 40%15s [-0.01 (-0.04, 0.02) pps/%MVT, *d* = -0.07 (-0.26, 0.11)] and control [0.01 (-0.01, 0.04) pps/%MVT, *d* = 0.07 (-0.08, 0.23)].

Peak discharge rates

A time by condition interaction was observed [β = -0.50 (-0.62, -0.37) pps, SE = 0.06; t = -7.64]. Peak discharge rates decreased from before to after the intervention only on control [-0.29 (−0.38, -0.21) pps, *d* = -0.52 (-0.67, -0.36)] and increased on 80%15s [0.20 (0.11, 0.29) pps, *d* = 0.35 (0.19, 0.51)] condition, but remained unchanged on 40%15s [0.06 (-0.04, 0.16) pps, *d* = 0.10 (-0.08, 0.28)] and 40%30s [-0.09 (-0.19, 0.02) pps, *d* = -0.16 (-0.34, 0.03)].

Recruitment thresholds

A time by condition interaction effect was observed [β = -0.89 (-1.17, -0.60) % of peak torque, SE = 0.15; t = -6.01]. Recruitment thresholds increased from before to after the intervention only on 40%15s [0.51 (0.29, 0.73) % of peak torque, *d* = 0.42 (0.24, 0.60)] and 40%30s [0.31 (0.09, 0.54) % of peak torque, *d* = 0.26 (0.07, 0.44)] conditions, and decreased on control [-0.38 (-0.57, -0.19) % of peak torque, *d* = -0.31 (-0.47, -0.16)] condition, but remained unchanged on 80%15s [-0.06 (-0.26, 0.14) % of peak torque, *d* = -0.05 (-0.21, 0.11)] condition.

Derecruitment thresholds

A time by condition interaction effect was observed [β = 0.53 (0.26, 0.79) % of peak torque, SE = 0.14; t = 3.89]. Derecruitment thresholds decreased from before to after the intervention only on 40%15s [-0.68 (-0.88, -0.48) % of peak torque, d = -0.61 (-0.79, -0.43)] and 80%15s [-0.37 (-0.55, -0.19) % of peak torque, d = -0.33 (-0.49, -0.17)] conditions, but remained unchanged on control [−0.15 (−0.33, 0.02) % of peak torque, d = -0.14 (-0.29, 0.02)] and 40%30s [-0.15 (−0.36, 0.05) % of peak torque, d = -0.14 (-0.32, 0.04)] conditions.

Table 2 describes the before and after estimated marginal means and mean differences for each MU outcome and tested conditions.

Table 2. Estimated marginal means and mean differences (95% confidence interval lower and upper limits) for ΔF, peak discharge rates, recruitment thresholds, brace height, and attenuation slope for handgrip and control conditions.

	ΔF (pps)	Brace height (% rTri)	Attenuation slope (pps/%MVT)	Peak discharge rate (pps)	Recruitment thresholds (% of peak torque)	Derecruitment thresholds (% of peak torque)
Control						
Before	5.29 (4.90, 5.69)	38.2 (36.1, 40.3)	0.40 (0.36, 0.44)	17.2 (16.1, 18.2)	8.54 (7.16, 9.92)	7.60 (6.64, 8.46)
After	5.22 (4.82, 5.62)	38.1 (36.0, 40.2)	0.41 (0.38, 0.45)	16.9 (15.8, 17.9)	8.16 (6.79, 9.54)	7.45 (6.59, 8.31)
After-before	-0.07 (−0.21, 0.06)	-0.11 (−1.84, 1.61)	0.01 (−0.01, 0.04)	**-0.29 (−0.38, −0.21)**	**-0.38 (−0.57, −0.19)**	-0.15 (-0.33, 0.02)
40%15s						
Before	5.64 (5.22, 6.05)	39.3 (37.0, 41.6)	0.40 (0.36, 0.44)	17.1 (16.0, 18.1)	8.08 (6.66, 9.49)	8.10 (7.23, 8.98)
After	5.73 (5.32, 6.15)	41.2 (38.9, 43.4)	0.39 (0.35, 0.42)	17.1 (16.1, 18.2)	8.58 (7.17, 10.00)	7.42 (6.54, 8.30)
After-before	0.10 (−0.06, 0.26)	1.86 (−0.15, 3.87)	−0.01 (−0.04, 0.02)	0.06 (−0.04, 0.16)	**0.51 (0.29, 0.73)**	**-0.68 (-0.88, -0.48)**
40%30s						
Before	5.49 (5.07, 5.91)	35.4 (33.1, 37.7)	0.46 (0.42, 0.49)	17.4 (16.3, 18.4)	8.32 (6.91, 9.74)	7.49 (6.61, 8.36)
After	5.81 (5.39, 6.24)	37.7 (35.4, 39.9)	0.41 (0.37, 0.45)	17.3 (16.2, 18.3)	8.63 (7.22, 10.05)	7.33 (6.45, 8.21)
After-before	**0.33 (0.16, 0.49)**	**2.24 (0.18, 4.30)**	**−0.05 (−0.08, −0.01)**	-0.09 (−0.19, 0.02)	**0.31 (0.09, 0.54)**	-0.15 (-0.36, 0.05)
80%15s						
Before	5.46 (5.05, 5.86)	37.3 (35.2, 39.5)	0.412 (0.38, 0.45)	17.4 (16.4, 18.5)	9.00 (7.61, 10.40)	8.08 (7.21, 8.94)
After	5.69 (5.29, 6.10)	39.8 (37.6, 42.0)	0.39 (0.35, 0.42)	17.6 (16.6, 18.7)	8.94 (7.55, 10.33)	7.71 (6.84, 8.58)
After-before	**0.24 (0.09, 0.38)**	**2.45 (0.64, 4.25)**	**−0.03 (−0.06, −0.00)**	**0.20 (0.11, 0.29)**	-0.06 (−0.26, 0.14)	**-0.37 (-0.55, -0.19)**

ΔF, Δ frequency; % rTri, percentage of the right triangle; MVT, maximum voluntary torque. Bold values indicate significant changes (95%CI not including zero).

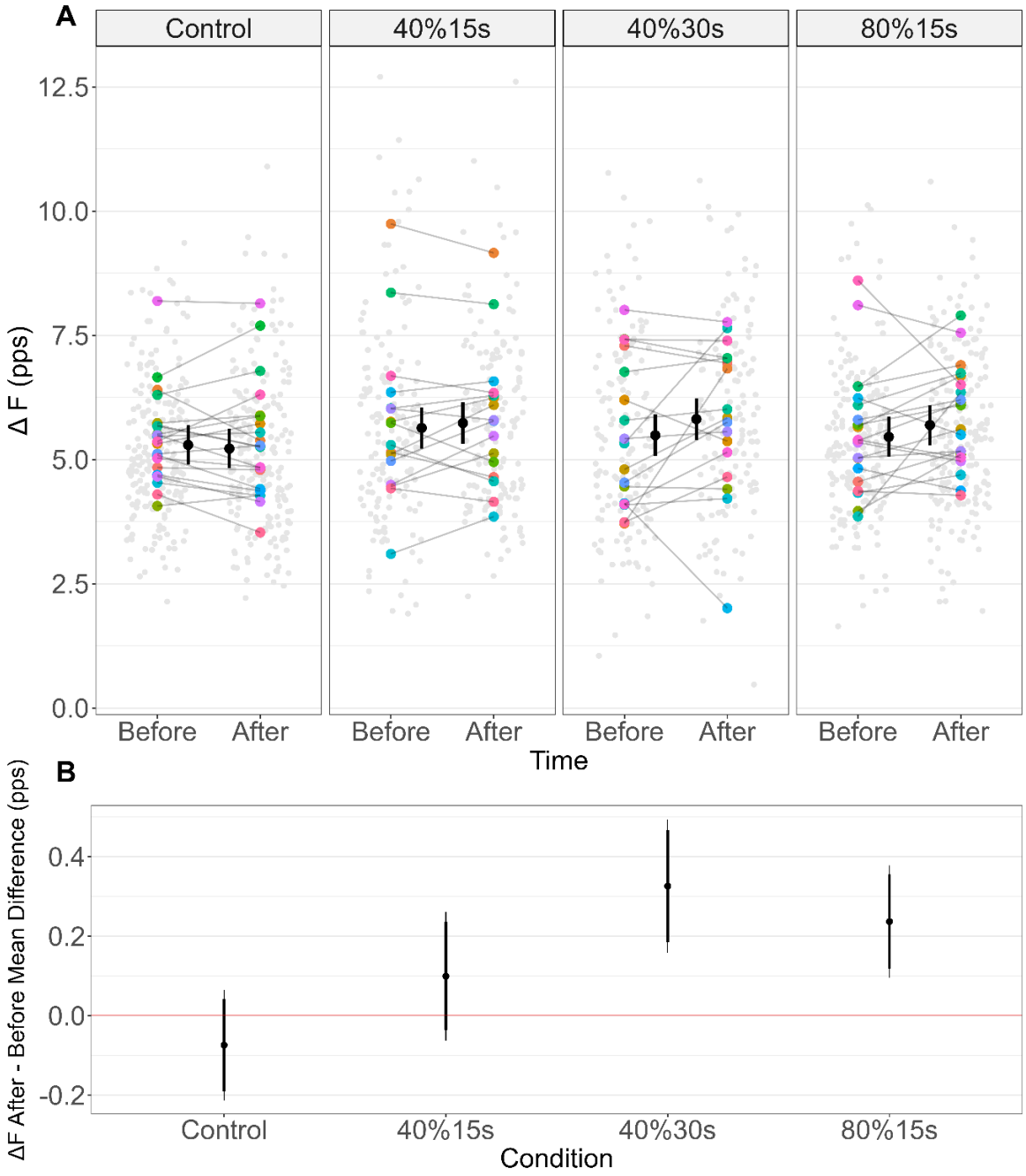

Figure 11A. The estimated marginal means (black circles) and respective 95% confidence intervals are offset to the middle.

Individual data points (averaged ΔF per participant) are coloured by participants and individual test units value are plotted in light grey. pps, pulses per second. ΔF remained unchanged before and after Control and 40%15s conditions but increased at 40%30s and 80%15s.

Figure 11B. Significant increases were observed on the handgrip at 40%30s and 80%15s conditions (not crossing the "zero" red line).

The thick inner line and the thin outer line represent the 90% and 95% confidence intervals, respectively.

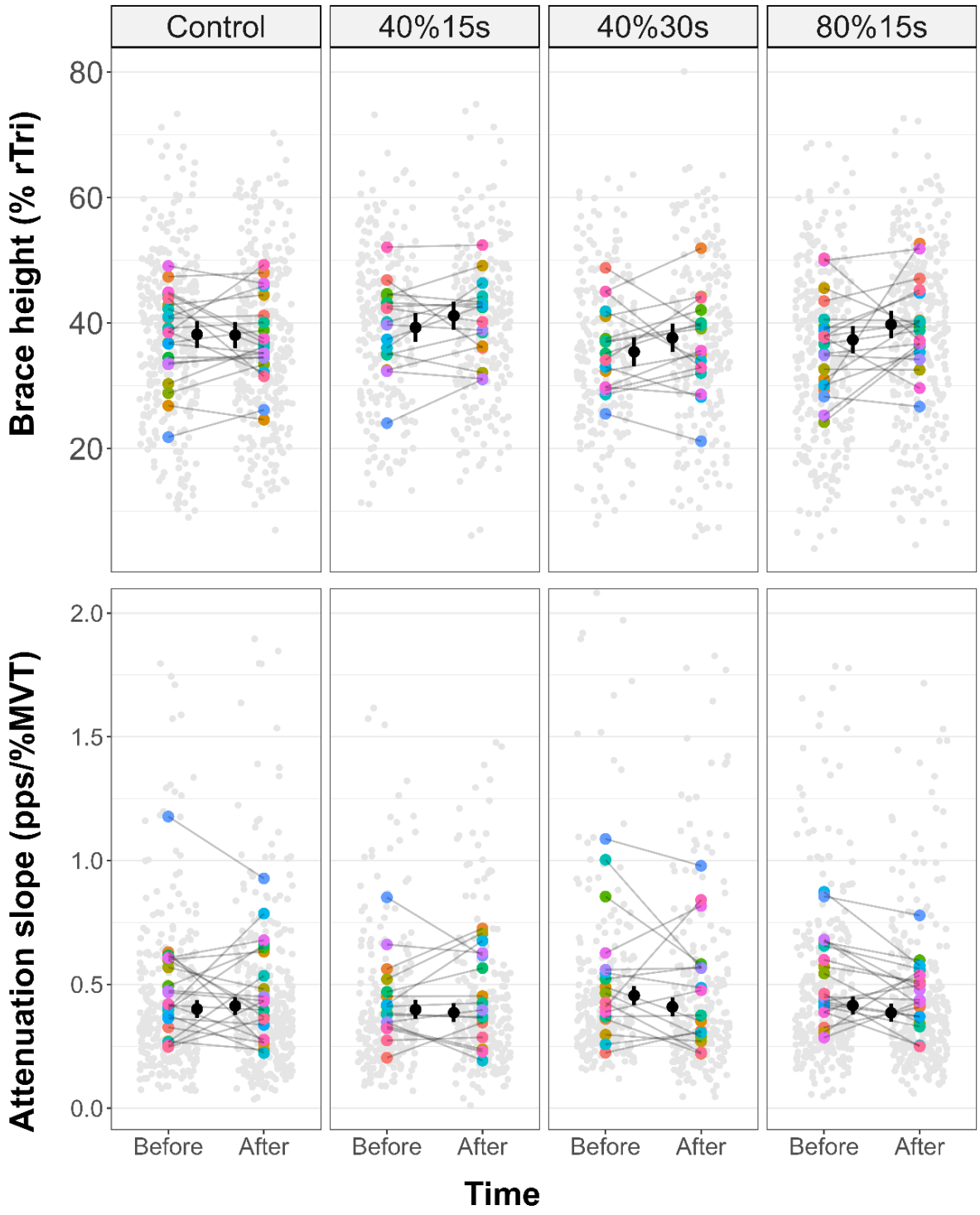

Figure 12. The estimated marginal means (black circles) and respective 95% confidence intervals are offset to the middle.

Individual data points (averaged brace height and attenuation slope per participant) are coloured by participants and individual motor units value are plotted in light grey. pps, pulses per second; MVT, maximum voluntary torque; % rTri, percentage of the right triangle. Both brace height and attenuation slope remained unchanged before and after Control and 40%15s conditions but, respectively, increased and decreased at 40%30s and 80%15s. Note: the y-axis for attenuation slope has been limited to 2 pps/%MVT to enhance the visualization of data points and their 95% confidence intervals. A full-range version of the figure is available at https://github.com/lugliara/PICs.

Discussion

We investigated whether remote handgrip contraction characteristics—duration (40%15s vs 40%30s), intensity (40%15s vs 80%15s), and the impulse (40%30s and 80%15s vs 40%15s)— affect tibialis anterior PICs. Quasi-geometric analyses estimated neuromodulatory (i.e., brace height) and inhibitory (i.e., attenuation slope) contributions to PIC responses. The main findings were: 1) ΔF increased after the higher handgrip impulse conditions; 2) brace height also increased under the same conditions; 3) attenuation slope consistently decreased. These findings suggest mechanical impulse of a remote contraction was the primary driver of increased ΔF, likely via enhanced neuromodulation and shifted inhibitory pattern.

Changes in ΔF, brace height, and attenuation slope between the two highest handgrip impulse conditions (80%15s and 40%30s) versus 40%15s and Control support our hypothesis, suggesting an impulse threshold might be necessary to induce tibialis anterior MU recruitment-derecruitment hysteresis. The small ΔF increases at the 40%30s (d = 0.47) and 80%15s (d = 0.34) conditions were similar to Mackay et al., (2023) in soleus (d = 0.30) and Orssatto et al., (2022) in tibialis anterior (d = 0.55), both using a similar 40%30s protocol. Such ΔF changes can be influenced by neuromodulatory and/or inhibitory inputs (Beauchamp et al., 2023). Monoamines are the main driver of PIC activity. In animals, voltage-clamp experiments show PICs result from voltage-gated L-type Ca^{2+} (Eckert & Lux, 1976; Svirskis & Hounsgaard, 1997) and Na^{+} (Harvey et al., 2006; Schwindt & Crill, 1977) channel activation. 5-HT and NE activating specific G-protein coupled receptors at motoneurons' dendrites and soma trigger intracellular signalling cascades, modulating these channels (Harvey et al., 2006; Heckman et al., 2009; Perrier & Hounsgaard, 2003). In humans, oral amphetamine, which is assumed to enhance the presynaptic NE release, increased estimates of ΔF (Udina et al., 2010), suggesting similar neuromodulation to that observed in animals in humans. Wei et al. (2014) showed 5-HT modulates motoneurons input-output gain via reuptake inhibitors and receptor blockers. Recently, multi-channel electromyography (EMG) during voluntary ramped contractions demonstrated decreased MU excitability with 5-HT blockers, measured via ΔF and/or other PIC estimates (Goodlich et al., 2023, 2024). Furthermore, higher ΔF has been observed at higher-intensity ramped contractions (Mackay et al., 2023; Orssatto et al., 2021; Škarabot et al., 2025), suggesting greater voluntary muscle activity in humans

is associated with higher PIC activity and, likely, higher 5-HT and NE in the spinal cord. Therefore, the ΔF increase observed in this study after higher-impulse handgrip contractions was likely driven by a transient monoamine raise; however, this mechanism was not directly tested and future studies with drugs affecting monoamines concentration might be able to address this mechanism.

We used brace height to estimate neuromodulatory contributions to motoneuron firing, as it was proposed to reflect increased serotonergic and/or noradrenergic input (Beauchamp et al., 2023). Thus, increases in brace height at 40%30s (*d* = 0.20) and 80%15s (*d* = 0.22) suggest remote handgrip contraction induced greater neuromodulation. In addition, PICs are highly sensitive to inhibition (Hultborn et al., 2003; Kuo et al., 2003), which controls unwanted movement that could arise from diffuse monoaminergic descending projections (Heckman et al., 2009). Spinal inhibition has been observed with small antagonist muscles length changes (Hultborn et al., 2003; Hyngstrom et al., 2007), tendon vibration (Matthews, 1966; Orssatto et al., 2022; Pearcey et al., 2022), passive muscle stretching (Trajano et al., 2014), and voluntary co-contraction (Gomes et al., 2024), likely via disynaptic inhibitory circuits activated by muscle spindle Ia afferents (Crone et al., 1987; Kuffler et al., 1951). However, the consistency of ramp-shaped contractions used in the present study before and after remote tasks make Ia-mediated changes unlikely. Inhibition input might also originates at supraspinal or segmental mechanisms, e.g., Renshaw cells (Hultborn & Pierrot-Deseilligny, 1979) or nociceptive inputs (Heckman et al., 2009). We used attenuation slope to estimate inhibitory input influence since inhibition patterns might predict this measure (Beauchamp et al., 2023). The small and trivial decrease in attenuation slope at 40%30s (*d* = -0.27) and 80%15s (*d* = -0.17) might indicate a transition from a tonic inhibitory pattern to inhibitory commands that are more reciprocal to excitation (i.e., push-pull excitation-inhibition synaptic control) (Škarabot et al., 2025). However, caution is needed, as ΔF sensitivity to inhibition patterns depends on neuromodulation levels(Beauchamp et al., 2023). Additionally, MUs exhibiting larger brace height theoretically show lower attenuation slope (Mesquita et al., 2024). Thus, our findings suggest that, once a certain impulse threshold is reached, remote contractions of either lower or higher intensity/duration similarly increase in the estimated PIC contribution to motoneuron self-sustained firing (ΔF). This effect is likely driven by increased neuromodulation onto the spinal cord, as

indicated by the small brace height increase, while attenuation slope might reflect a shift in inhibitory input patterns.

Complementarily, we investigated peak discharge rates and recruitment threshold because both are associated with motoneuron excitability and might also be influenced by PIC activity (Mesquita et al., 2024; Orssatto et al., 2022). Moderate and small decreases in peak discharge rates (d = -0.52) and recruitment threshold (d = -0.38) were observed in Control, contrasting with previous studies reporting no changes in tibialis anterior (Orssatto et al., 2022) or soleus (Mackay Phillips et al., 2023). Unlike those studies, participants in our study performed three randomised handgrip tasks with 5-minute intervals, possibly reducing carryover effects on ΔF but not on peak discharge rates or recruitment threshold. Furthermore, a small increase in peak discharge rates (d = 0.35) occurred only in 80%15s, while recruitment threshold increased in 40%15s (d = 0.42) and 40%30s (d = 0.26). Previous studies reported small peak discharge rates increases under 40%30s (Orssatto et al., (2022): d = 0.36; Mackay Phillips et al., (2023): d = 0.37), but while Orssatto et al. (2022) observed no changes in recruitment threshold, Mackay et al. (2023) did not report recruitment threshold results. Decreases in derecruitment thresholds were observed only in 40%15s (d = -0.61) and 80%15s (d = -0.33). The cause for non-alignment with ΔF is unclear; however, since both recruitment and derecruitment thresholds rely on highly variable torque data, these variables should be interpreted cautiously. These findings suggest that PICs differentially modulate ΔF, peak discharge rates, and recruitment and derecruitment thresholds, each providing distinct insights into motoneuron excitability.

Final considerations and future directions

This study combined robust non-invasive methods to estimate motor task influence on PIC activity in humans, likely via increased 5-HT input to the spinal cord. Using an innovative EMG protocol we non-invasively assessed key metrics of motoneuron excitability (e.g., ΔF, brace height and attenuation slope) without pharmacological intervention (Beauchamp et al., 2023). We also minimised the potential for type I error through rigorous statistical analysis (Yu et al., 2022). On the other hand, we assessed tibialis anterior MUs recruited during a ramp-shaped contraction to 20% of maximal force, consistent with previous studies investigating the

influence of remote contraction on ΔF (Mackay Phillips et al., 2023; Orssatto et al., 2022). This intensity is known to respond well to remote handgrip contractions and yield a higher number of motor units compared to higher contraction intensities (Orssatto et al., 2021). Thus, caution should be taken when extrapolating our findings to higher-threshold units, at higher contraction intensities contractions, and from different muscle groups. Additionally, direct in vivo 5-HT measurements remain technically impossible, so indirect methods are necessary. Lastly, while this study focused on 5-HT and NE contributions to PICs, other neuromodulators should be considered in future studies, e.g., spinal interneurons modulating motoneuron excitability via muscarinic m2 receptors , affecting resting K^+ conductance (Miles et al., 2007).

Future studies could combine EMG with additional methods to clarify findings. For instance: (1) since both 40%30s and 80%15s conditions increased ΔF in young adults, similar protocols could assess neuromodulatory capacity in populations with potentially reduced 5-HT input or impaired PIC activation, such older adults, individuals with neurodegenerative diseases, or partial spinal cord injuries; (2) further investigations are desirable whether impulse-specific effects on PIC modulation differ across muscle groups, as variations in motoneuron distribution may affect responsiveness to remote contraction-induced neuromodulation; (3) although remote handgrip contractions significantly increased ΔF of tibialis anterior MUs, the functional significance for motor control and force production remains unclear. Recent work has suggested within-subject associations between changes in ΔF and physical function following resistance training in older adults (Orssatto et al., 2023), and after short-term unloading and active recovery in young adults (Martino et al., 2024). Also, between-subject correlation of ΔF and physical function levels were observed in older adults (Orssatto et al., 2025), Even though there is evidence of a potential functional significance of ΔF levels on physical function, it is unclear whether the acute ΔF facilitation elicited by our experiment is sufficient to change motor output. Thus, further investigation into the functional role of acute ΔF modulation is warranted.

Conclusion

This study provides novel insights into the mechanisms through which remote voluntary muscle contractions influence motoneuron excitability, estimated by ΔF in

tibialis anterior motor units. By manipulating both the intensity and duration of a handgrip task, we identified contraction impulse as a critical factor driving changes in motoneuron recruitment-derecruitment hysteresis, which likely reflects in increased PICs activity. Specifically, higher ΔF values in remote task conditions with matched contraction impulse suggest that both higher and lower intensity/duration conditions can lead to increased PIC modulation, provided that a sufficient impulse threshold is met. The increased brace height observed in the impulse-matched conditions support our hypothesis that increased tibialis anterior ΔF following handgrip remote contraction may result from increased neuromodulation, possibly by augmented serotonergic input onto the spinal cord.

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Appendices

Consent form

QUT	**CONSENT FORM FOR QUT RESEARCH PROJECT**

The effect of isometric handgrip contraction on tibialis anterior motor neurone excitability

QUT Ethics Approval Number 6770

Research team contacts

Mr Lucas C Ugliara	07 3138 7395	camposug@qut.edu.au
Dr Lucas B R Orssatto	07 3138 3047	l.betdarosaorssatto@qut.edu.au
Dr Gabriel S Trajano	07 3138 5869	g.trajano@qut.edu.au

Statement of consent

By signing below, you are indicating that you:

- Have read and understood the information document regarding this project.
- Have had any questions answered to your satisfaction.
- Understand that if you have any additional questions you can contact the research team.
- Understand that you are free to withdraw at any time during the testing session and also to request the withdrawal of your data up to seven days after your participation.
- Understand that if you have concerns about the ethical conduct of the project you can contact the Research Ethics Advisory Team on 07 3138 5123 or email humanethics@qut.edu.au .
- Consent to undertaking the study procedures outlined in the information sheet.
- Agree to participate in the project.
- Authorise the use of this data in different research projects

Are you interested in receiving your individualised results at the completion of the project?

☐ **NO** ☐ **YES** If yes, please provide your preferred contact details below so we can re-contact you at the completion of the project.

Name _____

Signature _____

Date _____

Phone _____

Email _____

Please return the signed consent form to the researcher.

E-mail used for recruiting

Email-Recruit
Subject Title:
Participate in a research study looking at the mechanism of muscle activation

Dear colleagues

I would like to invite adults aged 18-40 years to be a participant in my research. This study aims to investigate how the physiological increase of serotonin in the spinal cord induced by a simple handgrip task performed at different intensities and volume influence the neural excitability of motoneurones in the leg. Participation in this study will involve attending to one visit at QUT. See attached document for further information.

If you are interested in participating or have any questions, please contact me via email.

Please note that this study has been approved by the QUT Human Research Ethics Committee (approval number 6770).

Many thanks for your consideration of this request.

Lucas Campos Ugliara
Doctoral student
07 3138 7395
camposug@qut.edu.au

Lucas Bet da Rosa Orssatto
Postdoctoral researcher
07 3138 3047
l.betdarosaorssatto@qut.edu.au

Dr Gabriel Trajano
Principal investigator
07 3138 5869
g.trajano@qut.edu.au

School of Exercise and Nutrition Science, Faculty of Health, Queensland University of Technology

Recruitment flyer

PARTICIPATE IN RESEARCH

Information for Prospective Participants

The effect of isometric handgrip contraction on tibialis anterior motor neurone excitability

QUT Ethics Approval Number 6770

Research team contacts

Principal Researcher: Dr Gabriel S Trajano

Associate Researchers: Mr Lucas Campos Ugliara

Dr Lucas B R Orssatto

School of Exercise and Nutrition Sciences, Faculty of Health

Queensland University of Technology (QUT)

What is the purpose of the research?

The purpose of this research is to investigate the influence of different contraction levels and duration of handgrip task on the excitability of motor neurones that activate leg muscles.

Why are you looking for people like me?

The research team is inviting people aged 18–40 years to participate in this study. We are looking for people, which are i) free of musculoskeletal injuries (such as, arthrosis or arthritis, prostheses, and recent surgeries) that limits the muscular contractions in both upper and lower limbs of at least one side of the body; ii) free of medical conditions (*e.g.,* multiple sclerosis, spinal cord injuries, and heart and respiratory diseases); iii) not using medications that could influence the neuromuscular recruitment system (*e.g.*, β-blockers, serotonin reuptake inhibitors); iv) who has body mass index lower than 30 kg/m^2.

What will you ask me to do?

Participation in this study will involve attending to the Neurophysiology laboratory (QUT Kelvin Grove campus, O-block, A-wing, room 326) on **one** occasion for approximately 90 min. You will have your height and body weight measured. Body composition will also be measured with octopolar Bioelectrical Impedance Analysis (BIA), which is a painless and safe test. You will also be asked to perform two short maximal (100%, your maximal capacity) and four submaximal (20%, 40%, 60% and 80% of your maximal capacity) contractions of your tibialis anterior muscle (front part of your lower leg) and forearms muscles (handgrip task). Seated in the chair of a dynamometer (*i.e.,* an equipment that measures force), contractions will be performed with right or left side, while using electrodes to measure your muscle activity.

Are there any risks for me in taking part?

There are minimal risks of muscle soreness associated to muscle contractions during the assessments, and minimal risks of skin allergic reaction to sticky electrodes. It should be noted that if you do agree to participate you can withdraw from participation in the research project without comment or penalty at any time.

Are there any benefits for me in taking part?

There will be no clear benefit for participating in this research. However, this research project will provide important information regarding the mechanism of activation of muscles, what can be used in the future to develop new drugs to treat neural diseases (i.e., lateral amyotrophic sclerosis and Parkinson's) and to integrate exoskeleton systems to the human body.

Will I be compensated for my time?

No, but we would very much appreciate your participation in this research.

I am interested – what should I do next?

If you are interested in participating in this study, for details of the next step, please contact:

Social media advertisement

QUT

PARTICIPATE in Research

THE EFFECT OF ISOMETRIC HANDGRIP INTENSITY ON MOTONEURON EXCITABILITY IN YOUNG ADULTS

Who we are looking for

- 18 to 40 years old
- BMI < 30 kg/m2
- No injuries in the limbs
- Not taking medication

Your role

- Participate voluntarily
- Attend a single visit (~2h)
- Perform strength test
- Perform body composition test

Where

QUT - Kelvin Grove

For more information:

Contact us QUT Kelvin Grove campus, O-block, A-wing, room 326 | 0410 309 324 camposug@qut.edu.au

Approved by the University Human Research Ethics Committee #6770

Information provided to participants prior their participation

PARTICIPANT INFORMATION FOR QUT RESEARCH PROJECT

The effect of isometric handgrip contraction on tibialis anterior motor neurone excitability

QUT Ethics Approval Number 6770

Research team

Principal Researcher: Dr Gabriel S Trajano
Associate Researchers: Mr Lucas C Ugliara
Dr Lucas B R Orssatto
School of Exercise and Nutrition Sciences, Faculty of Health
Queensland University of Technology (QUT)

Why is the study being conducted?

The purpose of this research is to investigate the influence of different contraction levels and duration of handgrip task on the excitability of motor neurones that activate leg muscles. This study outcome will give us important information on how humans control movements and muscle force levels.

What does participation involve?

Participation in this study will involve attending the Neurophysiology laboratory (QUT Kelvin Grove campus, O-block, A-wing, room 326) on **one** occasion for approximately 90 min. During this testing session, you will be asked to perform short maximal contractions (100% of your maximal capacity) for approximately 3 s using your hand and leg muscles. This will be followed by brief submaximal handgrip contractions (40% and 80% of your maximal capacity) and contractions of your lower leg (20% of your maximal capacity)), while using electrodes to measure muscle activity. The session will take approximately 90 min of your time.

We first need to confirm that you are eligible to take part:

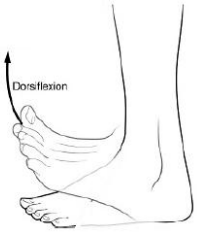
For this study we need the help of people aged from 18 to 40 years who are in good health, i) have a body mass index (BMI) <30 kg/m^2; ii) free of musculoskeletal injuries in limbs of at least one side of the body (such as arthrosis or arthritis, prostheses, and recent surgeries) that limits the muscular contractions; iii) free of medical conditions (e.g. multiple sclerosis, spinal cord injuries, and heart and respiratory diseases); iv) not using medications that could result in dizziness or antidepressants. This study will be completed at QUT Kelvin Grove and requires 30 volunteers.

Antropometric measures and body composition:

You will have your height and body weight measured. Body composition will also be measured with octopolar Bioelectrical Impedance Analysis (BIA), which is a painless and safe test.

Neuromuscular assessments:

During muscle contractions, muscle activation will be recorded non-invasively using sticky electrodes placed over your right or left tibialis anterior muscle (front part of your lower leg). To improve the electrode contact, the area will be shaved and cleaned with alcohol. Muscle strength and activation will be measured during two or three sub-maximal contractions for 15 or 30-s at 40% and 15-s at 80% of your maximal force using both feet and arms (ankle and handgrip). You will have 60-s to five minutes of rest time between contractions. These types of neuromuscular tests are widely used in a variety of populations and have a low risk for their health, and no discomfort is expected from the sticky



electrodes.

Figure 1. Handgrip and ankle dorsiflexion static contractions.

What are the possible benefits for you if you take part?

If you participate in this study, we can provide an objective measurement of strength of the muscles tested and body composition report. This research project will also provide important information regarding the mechanism of activation of muscles, what can be used in the future to develop new drugs to treat neural diseases (*i.e.,* lateral amyotrophic sclerosis and Parkinson's) and to integrate exoskeleton systems to the human body.

What are the possible risks for you if you take part?

The research team has identified, managed, and migrated the potential risks of this study. These include:

Risk: Muscles soreness due to the muscle strength testing.

Management: This is unlikely to occur since proper warm-up will be provided and the contractions are brief and isometric, what minimize the risk, but will be confirmed via the pre-exercise screening form completed during the initial familiarisation session. If the unlikely event injury occurs, QUT Security (3138 8888) will be informed, and QLD Emergency Services (000) (ambulance) will be contacted immediately. If anything occurs that prevents you from safely travelling home by yourself, the researcher will assist in organizing a taxi.

Risk: The sticky gel electrodes used during neuromuscular testing could cause a slight allergic reaction (rash).

Management: Sites will be correctly prepared before placing the electrodes.

Physical assessment procedures may have side effects. You may have none, some, or all the effects listed above, and they may range in severity. If you have any of these side effects, or are worried about them, speak to the researcher. The researcher will also be looking out for side effects during the interventions visits and if you start to feel any side effect after the completion of the visit. In any case, you can contact the researchers by phone or email (full contact is in the last page). Furthermore, there may be side effects that the researchers do not expect or do not know about and that may be serious. Please tell the researcher immediately about any new or unusual symptoms that you get. Many side effects will go away shortly after the assessment ends. Remember you can withdraw from the study at any time with no repercussions.

What about privacy and confidentiality?

All information provided to or collected by the research team will be treated confidentially. If you do not meet the inclusion criteria, and if requested, all collected data will be immediately destroyed to protect your privacy. You will not be identified in any report of the findings of the present investigation.

You are free to withdraw from this study at any time during the testing session and also to request the withdrawal of your data up to seven days after your participation. If you do withdraw from this study, any data collected will be retained in a non-identifiable form, and if requested, it will be destroyed. Data will be used only for scientific purposes (articles, conference proceedings, and thesis' final document) with no individual identification. Your privacy will be kept before, during, and after the study completion. The information collected in this study may be used in future research, which is also about the function of nerves and muscle. However, no personal identifying details will be included in any further analyses.

How do I give my consent to participate?

We would ask you to sign a written consent form (enclosed) to confirm your agreement to participate.

What if I have questions about the research project?

If you have any questions or require further information, please contact the listed researcher:

Mr Lucas C Ugliara 07 3138 7395 camposug@qut.edu.au
Dr Lucas B R Orssatto 07 3138 3047 l.betdarosaorssatto@qut.edu.au

Dr Gabriel S Trajano 07 3138 5869 g.trajano@qut.edu.au

What if I have a concern or complaint regarding the conduct of the research project?

QUT is committed to research integrity and the ethical conduct of research projects. If you wish to discuss the study with someone not directly involved, particularly in relation to matters concerning policies, information or complaints about the conduct of the study or your rights as a participant, you may contact the QUT Research Ethics Advisory Team on 07 3138 5123 or email humanethics@qut.edu.au.

Thank you for helping with this research project. Please keep this sheet for your information.

Study protocol

PROTOCOL FOR QUT LOW-RISK RESEARCH PROJECT

The effect of isometric handgrip contraction on tibialis anterior motor neurone excitability

Research team

Principal Researcher: Dr Gabriel S Trajano
Associate Researchers: Mr Lucas C Ugliara
Dr Lucas B R Orssatto
School of Exercise and Nutrition Sciences, Faculty of Health
Queensland University of Technology (QUT)

1. Project outline

The purpose of this research is to investigate the influence of different contraction levels and duration of handgrip task on the excitability of motor neurones that activate leg muscles. This study outcome will give us important information on how humans control movements and muscle force levels.

2. Methods

2.1. Study design

This research project will recruit 30 apparently healthy adults from the community. They will be assessed during a single visit to compare the influence of different intensities and duration of handgrip contractions in the excitability of lower limb motor neurones. Participation in this study will involve attending to the Neurophysiology laboratory (QUT Kelvin Grove campus, O-block, A-wing, room 326) on **one** occasion for approximately 90 min. During this testing session, they will be asked to perform short maximal (100%, your maximal capacity) and submaximal (20%, 40%, and 80% of your maximal capacity) contractions of their tibialis anterior muscle (front part of your lower leg) and forearms muscles while using electrodes to measure their muscle activity.

2.2. Participants

Participants aged 18 to 40 years will be included in the study if they are free of neuromuscular or medical conditions, such as multiple sclerosis, spinal cord injuries, and heart and respiratory diseases, and do not use medications that could result in dizziness or antidepressants. Sample size was calculated based on the effect sizes of a study that investigated the influence of handgrip contraction on the excitability of tibialis anterior motoneurones [1] in young (18–35 years) adults and non-sarcopenic older (≥65 years) adults. An effect size F of 0.25 for one-way repeated-measures design resulted in a sample size of 28 participants.

Participants will be recruited from social media and email to students and staff from QUT. They will be informed their participation is voluntary. If they decide to participate, a copy of their signed consent form will be provided to them. They will be advised that their decision to participate or not participate in the study will not affect their relationship with QUT. They will be informed if they participate, they can withdraw from the study at any time.

Participants must be able to comprehend the requirements of the study, willing to sign the informed consent form and be willing to comply with all study procedures. If assessed not capable of providing their own consent, participants will not be recruited into the study. All eligible patients will be offered the opportunity to receive information about the study via participant information and consent form documents, and have any questions answered by an investigator or member of the study team as appropriate. The Principal Investigator or Associate-Investigator prior to the consent form being signed will address all the concerns and questions. If the investigator believes that a potential participant has not fully understood the requirements and risks of study participation, that investigator may request further assistance or, for the safety and wellbeing of the participant, exclude them from the study.

2.2.1. Approach/es to provision of information to participants and/or consent
The investigator's email address will be included on every participant information document and online advertisements, with interested/prospective participants, asked to contact investigators should they wish to participate in the study. The investigators will therefore not be actively collecting email addresses but rather receive email queries from prospective participants. These email addresses will then be stored in the investigator's email program (Microsoft Outlook), which is password protected to ensure participant contact details remain private. Additionally, recruitment information sheets detail that contact information will be used solely for the purpose of recruitment for the study. The computer on which email correspondence will occur (Mr Lucas Ugliara's computer) is located in QUT Kelvin Grove campus, O Block, A wing, Level 4 room A401. For an example of email correspondence see below:

(from prospective participant):
Hi Mr Lucas Ugliara,
I have seen your information flyer and I am interested in participating in your study and just wanted to send an email to express interest.
Kind regards,
(Participant Name)

(from investigator):
Hi (Participant Name)
Thanks very much for your expression of interest, please see attached for some more detailed information regarding the study (Participant information sheet and Consent Form and the Withdraw Consent form.
Please read the information sheet carefully, noting the inclusion criteria, unfortunately, some interested participants may be ineligible to participate in the study. Feel free to ask any questions at this point as well.

2.3. Measurements
A non-invasive, painless, and quick assessment will be conducted using high-density electromyography, followed by body mass, height and body composition measurements.

2.3.1. Estimates of persistent inward currents
Before the tests, a surface electrode will be placed over the skin of right or left tibialis anterior muscle. To improve the electrode contact, the area will be shaved and cleaned with alcohol. At each visit, estimates of persistent inward currents will be obtained via paired-motor unit analysis. Participants will perform static handgrip and dorsiflexion muscular contractions (10 s increase and 10 s decrease in contraction intensity) up to 20% of maximal torque amplitude value. During the contractions non-invasive surface HD-EMG will be recorded from a 64-channel electrode grid placed over the tested muscle. Before performing the test, participants will be familiarised to triangular contractions with a subjective force of 20% of their perceived maximal force. Thereafter, they will perform a warm-up of handgrip and dorsiflexion with 5-s submaximal static contractions, with a subjective force of 20%, 40%, 60% and 80% relative to their perceived maximal force. Two min after the warm-up, participants will perform two maximal static contractions to measure their maximal handgrip and dorsiflexion voluntary isometric force and EMG amplitude. Contractions will have a 3-s duration and a 2-min rest interval. Two min after the maximal contractions, participants will perform two or more submaximal slowly ramped static contractions with 20% of their maximal torque amplitude using the tibialis anterior muscle. A screen will present the torque trace with a triangular pathway (ramp up and ramp down) to be followed by the participant. If the participants fail to follow the triangular torque trace (e.g., abrupt torque production in the beginning, abrupt deactivation at the end of the triangle, or high variability in the

torque production), the attempt will be repeated. The ramped static contractions will be performed before and after an isometric handgrip voluntary contraction to 40% of their maximal torque amplitude for 15 or 30-s, or to 80% of their maximal for 15-s.

2.3.2. Body mass, height and body composition

Body mass and height will be measured with a scale and stadiometer, respectively. Body composition will be measured with octopolar Bioelectrical Impedance Analysis (BIA). The BIA analysis uses low-voltage current through the body to estimate body fat and lean body mass based on tissue resistance. This technic has been largely used before [2] and is considered safe even for patients with cardiac implantable electronic devices [3].

2.4. Data Analysis

HD-EMG signals will be decomposed off-line into single motor unit discharge events using a convolutive blind source separation MATLAB algorithm. This algorithm has been extensively validated against the standard intramuscular fine wire methods across a variety of muscles and contraction intensities [4, 5]. Estimates of persistent inward current will be quantified as ΔFs, which will be calculated as the change in discharge rate of a lower-threshold (control) motor unit from the moment of recruitment to the moment of de-recruitment of a higher-threshold (test) unit.

All data will be compiled into an excel spreadsheet for further analysis. Statistical analysis will be performed in R. Linear mixed models will be used to determine changes in ΔF before and after intervention (i.e., handgrip) or control conditions. Also, intra-class correlation coefficient, standard error of measurement, and coefficient of variation will be calculated. Statistical significance will be considered at an alpha level of 5%.

2.5. Risks and management

The research team has identified, managed and migrated the potential risks of this study. These include:

Risk: Muscles soreness due to the muscle strength testing.

Management: This is unlikely to occur since proper warm-up will be provided and the contractions are brief and isometric, what minimize the risk, but will be confirmed via the pre-exercise screening form completed during the initial familiarisation session. If the unlikely event injury occurs, QUT Security (3138 8888) will be informed, and QLD Emergency Services (000) (ambulance) will be contacted immediately. If anything occurs that prevents you from safely travelling home by yourself, the researcher will assist in organizing a taxi.

Risk: The sticky gel electrodes used during neuromuscular testing could cause a slight allergic reaction (rash).

Management: Sites will be correctly prepared before placing the electrodes, which should minimise this risk.

Physical assessment procedures may have side effects. You may have none, some, or all of the effects listed above, and they may range in severity. If you have any of these side effects, or are worried about them, speak to the researcher. The researcher will also be looking out for side effects during the interventions visits and if you start to feel any side effect after the completion of the visit. In any case, you can contact any of the researchers by phone or email (full contacts are in the last page). Furthermore, there may be side effects that the researchers do not expect or do not know about and that may be serious. Please tell the researcher immediately about any new or unusual symptoms that you get. Many side effects will go away shortly after the assessment ends. Remember you can withdraw from the study at any time with no repercussions.

2.6. Confidentiality

Data will be collected in a potentially re-identifiable form in order to compare data and results between testing sessions. However, all participant data will be recorded and stored in de-identified form through the use of a code number. This code system will be decipherable by only the principal investigator and principal supervisor after using a linked code key sheet that will be stored separately to the de-identified results. The code will be developed from a combination of letters and numbers to construct each code. All participant data will be reported in a de-identified form when used in any presentations, publications or alternate academic outputs.

2.7. Plans for dissemination and publication of project outcomes

Firstly, the plan is to present the initial project outcomes in an abstract poster or oral presentation format in the Motoneuron Society Meeting 2024. Secondly, an article will be written with the results and submit to Q1 peer-reviewed journal for publication.

2.8. Other potential uses of the data at the end of the project

This data will be relevant for other research projects developed by our group. Therefore, the data from this project may be used as reference values for research projects testing different populations.

2.9. Data Management

The originals hard copy documents will be scanned and transformed in PDF files. So, every data will be stored in different documents (PDF and EXCEL). Additionally, the original documents and PDF files will be stored as hard copies.

The digital documents will be stored in Lucas Ugliara's QUT computer (linked to the QUT OneDrive), his personal computer, one personal password-protected external Hard-drive, and one password enables USB. The hard copies files will be stored in the QUT office.

3.0. References

1. Orssatto LBR, Fernandes GL, Blazevich AJ, Trajano GS (2022) Facilitation-inhibition control of motor neuronal persistent inward currents in young and older adults. bioRxiv. doi: 10.1101/2022.08.08.503135
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5. Enoka RM (2019) Physiological validation of the decomposition of surface EMG signals. J Electromyogr Kinesiol 46:70–83. doi: 10.1016/j.jelekin.2019.03.010

Withdrawal of consent form

WITHDRAWAL OF CONSENT FOR QUT RESEARCH PROJECT

The effect of isometric handgrip contraction on tibialis anterior motor neurone excitability

QUT Ethics Approval Number 6770

Research team contacts

Mr Lucas C Ugliara	07 3138 7395	camposug@qut.edu.au
Dr Lucas B R Orssatto	07 3138 3047	l.betdarosaorssatto@qut.edu.au
Dr Gabriel S Trajano	07 3138 5869	g.trajano@qut.edu.au

School of Exercise and Nutrition Sciences, Faculty of Health
Queensland University of Technology (QUT)

I hereby wish to WITHDRAW my consent to participate in the research project named above. I understand that this withdrawal WILL NOT jeopardise my relationship with QUT.

☐ Please use data collected about me so far for the study.

Name _____

Optional: Signature _____

Date signed or noted _____

Optional: Reason for withdrawal (if provided) _____

Please return the signed withdrawal form to the researcher.
Please note that you may also withdraw verbally or via email.

ParQ

2023 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ **OR** high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow Global Physical Activity Guidelines for your age (https://www.who.int/publications/i/item/9789240015128).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION

If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

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2023 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm

If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you currently have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes

If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

2023 PAR-Q+

6. **Do you have any Mental Health Problems or Learning Difficulties?** This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome

If the above condition(s) is/are present, answer questions 6a-6b — If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d — If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c — If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c — If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**

If you have other medical conditions, answer questions 10a-10c — If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

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2023 PAR-Q+

If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration.
The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

Key References

1. Jamnik VK, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the effectiveness of clearance for physical activity participation; background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.
3. Chisholm DM, Collis ML, Kulak LL, Davenport W, and Gruber N. Physical activity readiness. British Columbia Medical Journal. 1975;17:375-378.
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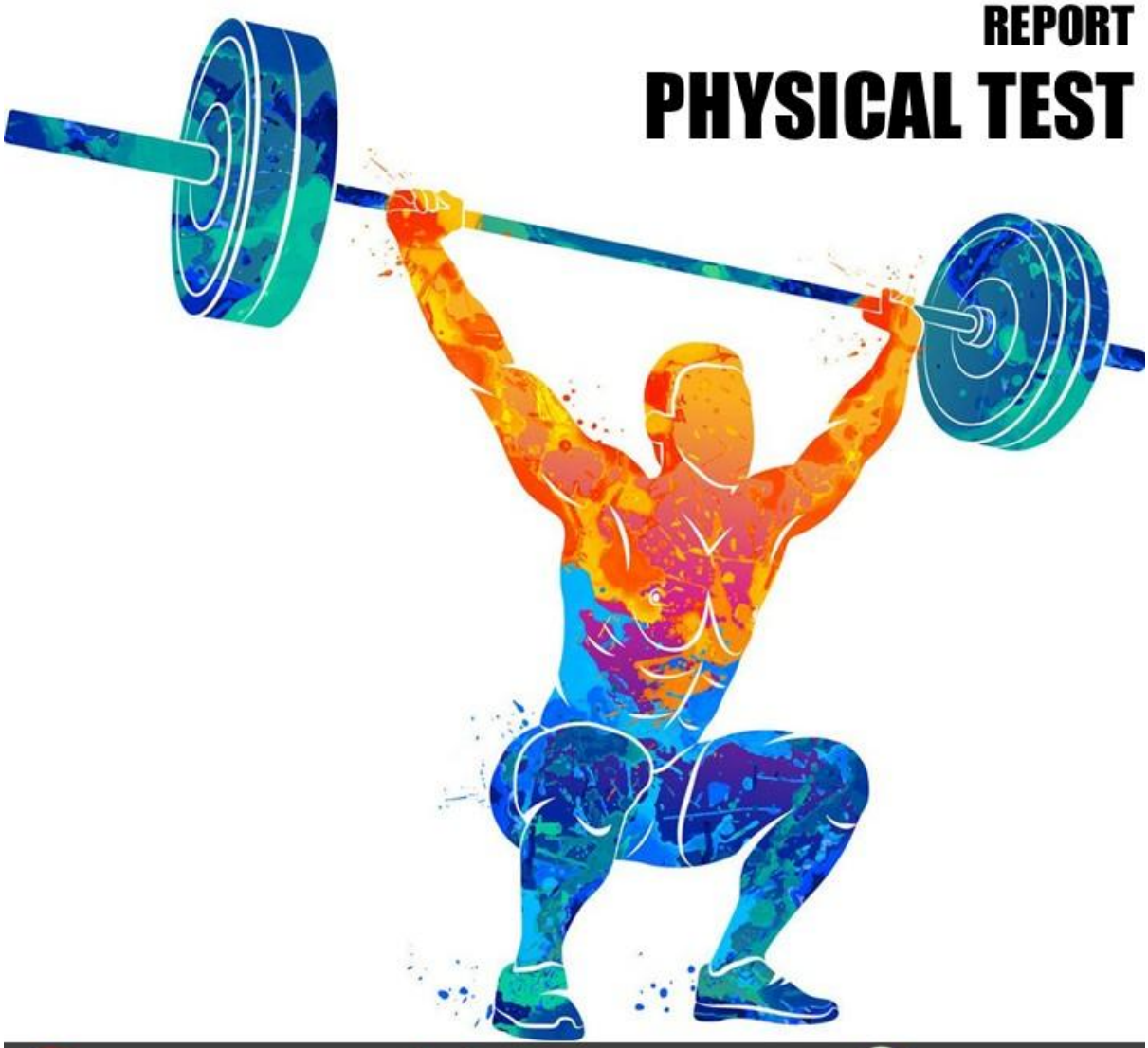
01-11-2022

Physical test report provided to the participants

Queensland University of Technology

REPORT

PHYSICAL TEST



qut.edu.au camposug@qut.edu.au 0410 309 324

QUT

PHYSICAL TEST

PERSONAL DATA

Name:		Date:		Age:	
Body mass (kg):		Height (m):		BMI (kg/m²):	

BODY COMPOSITION

Fat mass (%)		Free fat mass (kg)	
Trunk (%)		Trunk (kg)	
Arm R (%)		Arm R (kg)	
Arm L (%)		Arm L (kg)	
Leg R (%)		Leg R (kg)	
Leg L (%)		Leg L (kg)	
Total body water (%)		Visceral fat mass (kg)	
Basal Metabolic Rate (kj)		Basal Metabolic Rate (kcal)	

STRENGTH TEST

HANDGRIP (kg)	1st	
	2nd	
DORSIFLEXION (N·m)	1st	
	2nd	

NORMATIVE VALUES

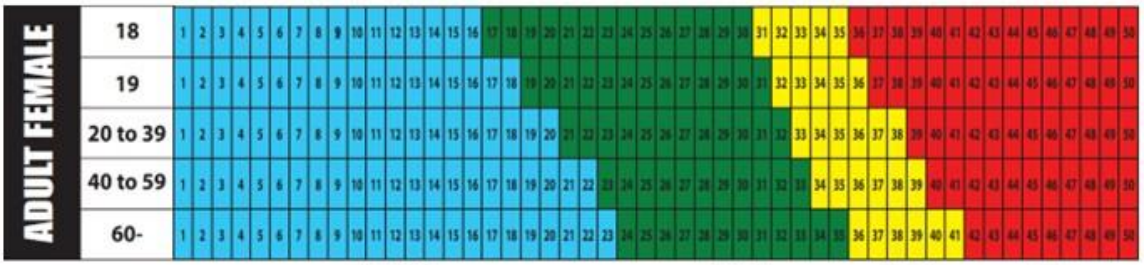
Age	Female Dorsiflexion (N·m)	Male Dorsiflexion (N·m)
5 – 9	8.2 (2.9)	5.6 (2.0)
10 – 14	16.6 (3.8)	15.8 (6.2)
15 – 19	23.8 (6.9)	37.5 (13.1)
20 – 29	23.9 (6.7)	39.5 (10.5)
30 – 39	24.4 (5.6)	34.3 (9.8)
40 – 49	25.0 (6.2)	39.8 (9.2)
50 – 59	23.4 (5.2)	39.7 (8.6)
60 – 69	21.3 (5.4)	31.6 (9.3)
70 – 80	21.4 (6.2)	39.5 (15.6)

doi: 10.1186/1471-2474-14-104

PHYSICAL TEST

NORMATIVE VALUES

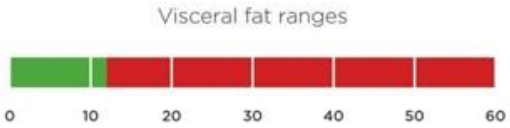
BODY FAT PERCENTAGE



Gallagher D et al. Am J Clin Nutr 2000; 72:694-701
"Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index."

Jebb S, McCarthy D, Fry T, Prentice AM (2004). New body fat reference curves for children.
Obesity Reviews (NAASO Suppl). A156

VISCERAL FAT



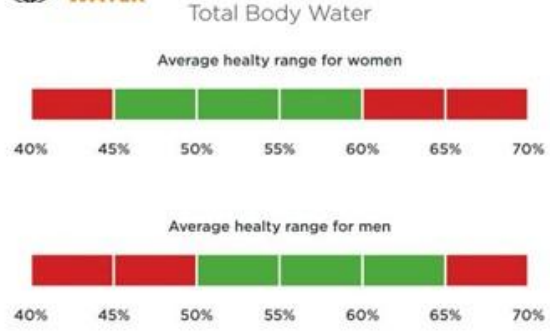
Healthy 1 - 12

Indicates you have a healthy level of visceral fat. Continue monitoring your rating to ensure it stays within the healthy range.

Excessive 12 - 59

Indicates you have an excess level of visceral fat. Consider making changes in your diet and/or increasing the amount of exercise you do.

TOTAL BODY WATER



QUT

PHYSICAL TEST

NORMATIVE VALUES

TABLE 1 SUMMARY OF HAND-GRIP STRENGTH MEASUREMENTS BY SIDE, SEX, AND AGE-GROUP STRATA*

				Percentile				
Hand/Sex/Age, y	Height, m	Weight, kg	Strength, kg	10	25	50	75	90
Dominant								
Male								
18-24 (n = 36)	1.81 ± 0.08	82.0 ± 16.9	47.0 ± 8.1	36.2	41.4	47.8	51.2	57.9
25-29 (n = 35)	1.78 ± 0.07	86.4 ± 21.9	49.7 ± 11.6	33.7	43.3	49.3	59.4	66.2
30-34 (n = 29)	1.75 ± 0.06	91.0 ± 17.9	46.5 ± 12.1	31.2	36.4	46.1	56.4	63.1
35-39 (n = 41)	1.77 ± 0.07	92.5 ± 22.1	47.1 ± 11.9	30.3	39.7	50.1	54.3	60.8
40-44 (n = 47)	1.75 ± 0.07	90.0 ± 19.6	46.7 ± 11.7	34.3	39.9	45.9	54.4	63.1
45-49 (n = 32)	1.73 ± 0.06	89.1 ± 17.9	42.8 ± 10.9	31.1	35.8	40.7	48.2	59.2
50-54 (n = 46)	1.78 ± 0.08	93.8 ± 17.2	44.0 ± 10.3	30.4	39.0	44.8	52.3	56.7
55-59 (n = 27)	1.77 ± 0.08	92.3 ± 22.4	40.7 ± 10.4	28.2	32.4	38.7	47.8	56.3
60-64 (n = 33)	1.77 ± 0.08	90.3 ± 13.4	38.4 ± 10.3	23.3	30.4	40.3	44.9	52.5
65-69 (n = 22)	1.74 ± 0.08	86.2 ± 17.9	36.8 ± 10.5	17.8	31.5	36.6	45.8	50.1
70-74 (n = 39)	1.75 ± 0.08	88.3 ± 18.0	34.7 ± 9.0	16.7	29.3	36.3	41.2	45.6
75-79 (n = 24)	1.76 ± 0.08	86.2 ± 14.0	32.7 ± 10.1	18.4	25.9	33.5	36.6	43.5
80-85 (n = 38)	1.75 ± 0.08	81.1 ± 13.0	28.1 ± 9.1	15.6	21.5	29.5	34.6	38.2
Female								
18-24 (n = 54)	1.61 ± 0.07	72.3 ± 21.2	28.1 ± 7.1	17.6	22.4	28.4	33.8	38.0
25-29 (n = 102)	1.61 ± 0.07	73.3 ± 20.1	29.6 ± 7.0	20.2	25.4	29.6	33.6	39.7
30-34 (n = 109)	1.63 ± 0.07	76.1 ± 19.6	28.9 ± 6.2	20.5	23.9	29.8	33.0	37.1
35-39 (n = 90)	1.62 ± 0.07	75.2 ± 17.4	29.2 ± 6.2	20.0	24.5	30.3	33.0	38.0
40-44 (n = 88)	1.63 ± 0.07	75.9 ± 18.4	29.9 ± 6.2	22.8	26.5	30.4	33.8	37.4
45-49 (n = 52)	1.63 ± 0.08	79.7 ± 19.1	28.8 ± 7.2	17.7	25.2	28.7	34.4	37.6
50-54 (n = 65)	1.63 ± 0.07	75.6 ± 16.0	28.2 ± 6.3	19.7	24.6	28.2	32.7	35.2
55-59 (n = 30)	1.62 ± 0.07	76.6 ± 16.2	25.1 ± 6.2	16.9	20.7	24.1	30.2	32.2
60-64 (n = 58)	1.62 ± 0.07	76.7 ± 17.4	23.6 ± 6.5	15.9	19.2	24.4	28.1	31.8
65-69 (n = 29)	1.62 ± 0.07	80.0 ± 21.5	22.1 ± 6.6	11.7	19.3	22.2	25.0	31.2
70-74 (n = 43)	1.60 ± 0.07	77.4 ± 18.8	21.5 ± 5.1	15.2	19.5	22.5	23.9	27.5
75-79 (n = 17)	1.58 ± 0.08	66.7 ± 10.4	19.6 ± 6.0	12.6	15.7	18.2	22.4	27.8
80-85 (n = 46)	1.60 ± 0.06	70.0 ± 11.3	19.9 ± 4.4	14.5	16.6	19.5	21.8	27.0

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