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Antagonism of 5-HT₂ receptors attenuates self-sustained firing of human motor units

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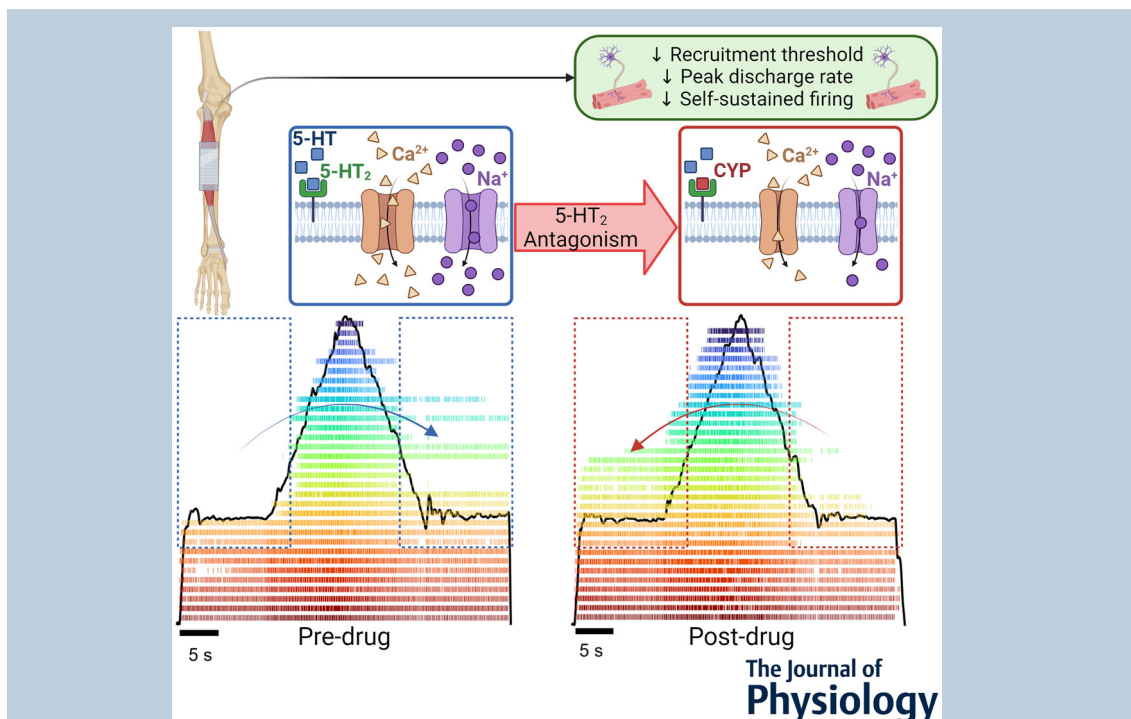
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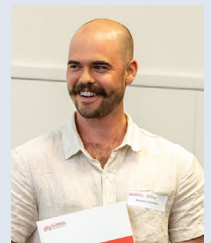
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Abstract 5-HT₂ receptors on motoneurons play a critical role in facilitating persistent inward currents (PICs). Although facilitation of PICs can enhance self-sustained firing after periods of excitation, the relationship between 5-HT₂ receptor activity and self-sustained firing in human

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motor units (MUs) has not been resolved. MU activity was assessed from the tibialis anterior of 10 healthy adults (24.9 ± 2.8 years) during two contraction protocols. Both protocols featured steady-state isometric contractions with constant descending drive to the motoneurone pool. However, one protocol also included an additional phase of superimposed descending drive. Adding and then removing descending drive in the middle of steady-state contractions altered MU firing behaviour across the motor pool, where newly recruited units in the superimposed phase were unable to switch off ($P = 0.0002$), and units recruited prior to additional descending drive reduced their discharge rates ($P < 0.0001$, difference in estimated marginal means (Δ) = 2.24 pulses/s). The 5-HT₂ receptor antagonist, cyproheptadine, was then administered to determine whether changes in MU firing were mediated by serotonergic mechanisms. 5-HT₂ receptor antagonism caused reductions in MU discharge rate ($P < 0.001$, $\Delta = 1.65$ pulses/s), recruitment threshold ($P = 0.00112$, $\Delta = 1.09\%$ maximal voluntary contraction) and self-sustained firing duration ($P < 0.0001$, $\Delta = 1.77$ s) after the additional descending drive was removed in the middle of the steady-state contraction. These findings indicate that serotonergic neuromodulation plays a key role in facilitating discharge and self-sustained firing of human motoneurons, where adaptive changes in MU recruitment must occur to meet the demands of the contraction.

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Abstract figure legend High-density surface electromyography (HDsEMG) was recorded from the tibialis anterior during two distinct contraction protocols. Both protocols featured steady-state isometric contractions with a constant descending drive to the motoneurone pool. However, one protocol also included an additional phase of superimposed descending drive. Adding and then removing the descending drive in the middle of steady-state contractions altered motor unit (MU) firing behaviour across the motor pool, where newly recruited units in the superimposed phase were unable to switch off. The 5-HT₂ receptor antagonist, cyproheptadine, was then administered to determine whether changes in MU firing were mediated by serotonergic mechanisms. 5-HT₂ receptor antagonism caused reductions in the MU discharge rate, recruitment threshold and self-sustained firing duration. These findings indicate that serotonergic neuromodulation plays a key role in facilitating the discharge and self-sustained firing of human motoneurons, where adaptive changes in MU recruitment must occur to meet the demands of the contraction.

Key points

- Animal and cellular preparations indicate that somato-dendritic 5-HT₂ receptors regulate the intrinsic excitability of motoneurons.
- 5-HT₂ receptor antagonism reduces estimates of persistent inward currents in motoneurons, which contribute to self-sustained firing when synaptic inputs are reduced or removed.
- This human study employed a contraction task that slowly increased (and then removed) the additional descending drive in the middle of a steady-state contraction where marked self-sustained firing occurred when the descending drive was removed.
- 5-HT₂ receptor antagonism caused widespread reductions in motor unit (MU) discharge rates during contractions, which was accompanied by reduced recruitment threshold and attenuation of self-sustained firing duration after the removal of the additional descending drive to motoneurons.
- These findings support the role that serotonergic neuromodulation is a key facilitator of MU discharge and self-sustained firing of human motoneurons, where adaptive changes in MU recruitment must occur to meet the demands of the contraction.

Introduction

Motoneurone responsiveness to descending synaptic inputs (corticospinal, reticulospinal, vestibulospinal),

interneuronal synaptic inputs, and afferent synaptic inputs can be regulated by intracellular signalling pathways initiated by neuromodulatory receptors on

motoneurons. Descending neuromodulation systems emerge from brainstem nuclei and contribute to the integration of synaptic input to the motoneuron in part via activating dendritic persistent inward currents (PICs). PICs are voltage-gated slow-activating L-type Ca²⁺ and fast-activating persistent Na⁺ currents which provide an additional intrinsic source of depolarising current to synaptic inputs to motoneurons (Hounsgaard & Kiehn, 1985, 1989; Hultborn et al., 2003; Li & Bennett, 2003; Powers & Binder, 2000; Schwindt & Crill, 1980). PICs are not only capable of amplifying initial firing rates (Bennett et al., 1998; Hounsgaard et al., 1988; Lee & Heckman, 1998a), but also promote self-sustained firing after the removal of synaptic inputs that depolarised the motoneuron (Hounsgaard et al., 1988; Lee & Heckman, 1998b). This is typically reflected by hysteresis in motor unit (MU) firing characteristics, where the derecruitment of motoneurons occurs at lower levels of synaptic input compared with the recruitment of motoneurons (Gorassini et al., 2002; Kiehn & Eken, 1997). As the natural state of the motoneuron is to keep firing once it is activated, cessation of self-sustained firing typically requires direct synaptic inhibition to deactivate the motoneuron PIC (Kuo et al., 2003).

A growing number of human investigations are reporting concomitant changes to estimates of PIC amplitude and MU discharge rate (Hassan et al., 2021; Mesquita et al., 2022; Orssatto et al., 2021), where serotonin (5-HT) has been heavily implicated in altering PIC activity. The serotonergic system originates in the raphe nuclei of the brainstem, and releases 5-HT onto motoneurons in the spinal cord via long descending monosynaptic projections. We have recently demonstrated that the 5-HT₂ receptor mediates MU discharge rates, where antagonism of 5-HT₂ receptor activity leads to reductions in discharge during rapid (Goodlich et al., 2022) and steady-state isometric dorsiflexions (Goodlich et al., 2023a). Moreover, reduced onset-offset hysteresis in MU firing (Delta F) accompanies 5-HT₂ receptor antagonism, whereby reductions in Delta F are typically interpreted as a suppression in PIC amplitude in human motoneurons (Goodlich et al., 2023a). Although these studies highlight that serotonergic neuromodulation influences MU behaviour, it is important to note that each of these 5-HT studies assessed MU firing for contraction tasks where force was developed from a resting muscle state. Although the raphe nuclei are tonically active during quiet rest, serotonergic drive is largely thought to be coupled to descending drive (Jacobs & Fornal, 1997; Jacobs et al., 2002; Veasey et al., 1995); meaning it was unlikely that high concentrations of 5-HT were released onto motoneurons prior to the commencement of motoneuron depolarisation. Thus, PICs may have been required to shift from a relatively inactive state

to a relatively more active state after depolarisation commenced from the addition of excitatory synaptic input. Converging lines of evidence suggest that excitatory synaptic input is needed to observe serotonergic effects in human spinal motoneuron excitability (Henderson et al., 2024; Thorstensen et al., 2022), so it is possible that the effects of serotonergic neuromodulation, and in particular self-sustained firing of motoneurons, will differ depending on the initial state of the motor system.

The purpose of this study was to examine how serotonergic neuromodulation contributes to the self-sustained firing of motoneurons in humans. To achieve this, we contrasted MU firing behaviour during two types of contraction protocol. Both protocols featured steady-state contraction phases where force was developed from rest. Thus, the descending drive to the motoneuron pool, inclusive of ionotropic and neuromodulatory synaptic input, was consistent. However, one of the protocols featured an additional phase where additional descending drive was superimposed on the steady-state contraction. In doing so we established that adding (and then removing) descending drive in the middle of steady-state contractions: (1) markedly suppresses MU discharge in already firing units, and (2) recruited new units in the superimposed phase that were unable to switch off due to their self-sustained firing. A pharmacological intervention was used to further examine synaptic input when the motor pool received the superimposed descending drive, where a 5-HT₂ receptor antagonist was used to determine whether the changes in discharge rate and self-sustained firing were mediated by a serotonergic mechanism. It was hypothesised that 5-HT₂ receptor antagonism would abolish changes in MU discharge rate and mitigate self-sustained firing arising from additional synaptic inputs, which would support a neuromodulation mechanism involving the 5-HT₂ receptor.

Methods

Participants and ethical approval

Twelve healthy, recreationally active, individuals volunteered for the study (age 24.9 ± 2.8 years, five female). Participants were screened prior to enrolment with a medical history questionnaire that contained exclusion criteria specific to acute or chronic neuromuscular injury, as well as the administration of cyproheptadine. Participants who routinely take antidepressants or other medications which directly influence central nervous system activity were excluded from the study. Participants were instructed to refrain from any stimulants or depressants such as caffeine, alcohol or moderate-to-high intensity exercise in the 12 h preceding testing. Approval for testing procedures were

obtained via Griffith University's Human Research Ethics committee (GU Ref No: 2023/153), and all procedures were performed in accordance with the *Declaration of Helsinki* except for registration in a database. Written informed consent was obtained for all participants prior to testing.

Drug administration

Control neurophysiological measurements were obtained before a single oral dose of cyproheptadine (8 mg). Two and a half hours following pill ingestion, post-drug testing took place where neurophysiological measurements were once again made. The timing of testing aligned with high plasma concentrations of cyproheptadine (D'Amico et al., 2013; Wei et al., 2014), as well as the testing window reported in previous cyproheptadine studies (Goodlich et al., 2022, 2023a; Thorstensen et al., 2021). The dosage of cyproheptadine is consistent with previous studies which

have used cyproheptadine as an intervention to assess serotonergic effects on the motor system (D'Amico et al., 2013; Goodlich et al., 2022, 2023a; Thorstensen et al., 2021; Wei et al., 2014). Cyproheptadine binds with high affinity to 5-HT_{2A/B/C} receptors (Boess & Martin, 1994; Honrubia et al., 1997), attenuating serotonergic effects via competitive antagonism. There were no adverse effects of cyproheptadine in the current study; however, low levels of drowsiness were reported by all participants ~ 4 h after pill ingestion.

Participant setup and torque measurement

Participants sat comfortably in a motorised therapy chair, which was adjusted for each participant to position their right hip and knee at 90° of flexion in the sagittal plane (Fig. 1A). Since a shortened agonist muscle can enhance self-sustained MU firing in humans (Beauchamp et al., 2023), the right foot was positioned at 10° of dorsiflexion

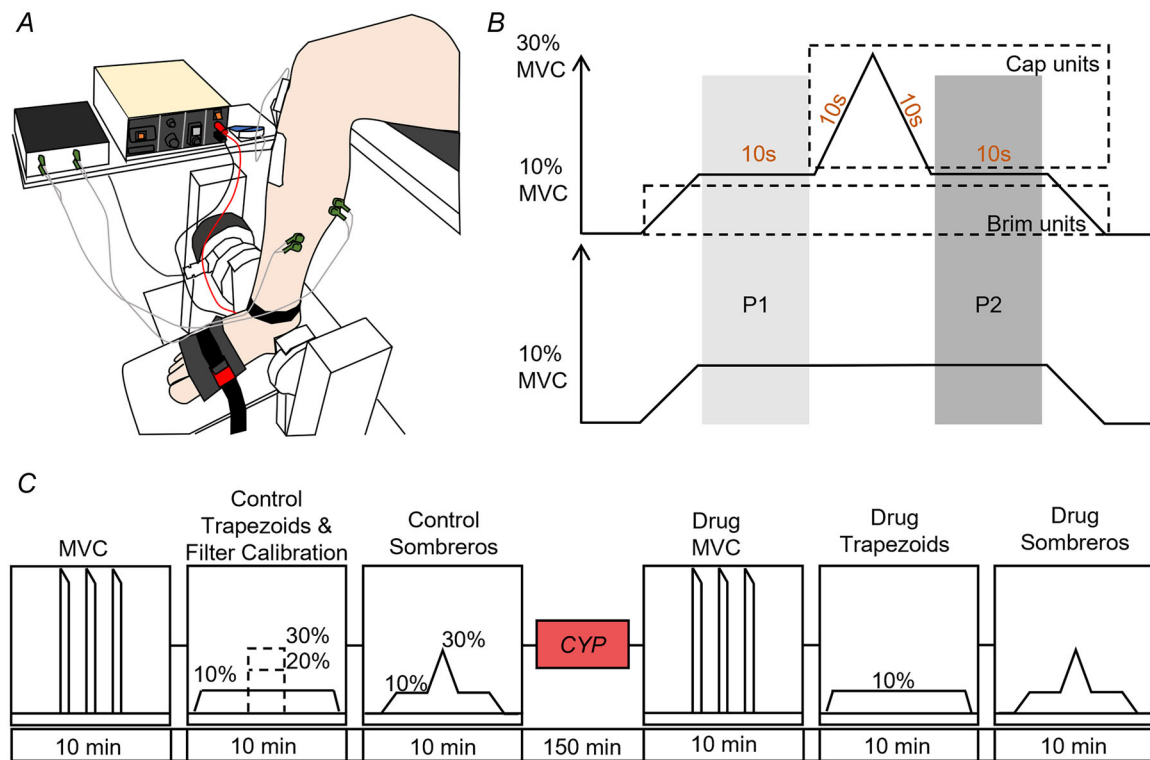


Figure 1. Participant set up and contraction protocol

A, the right foot of the participant was secured to a torque sensor and HDsEMG electrodes were fixed over the tibialis anterior muscle belly. B, trapezoid and sombrero-shaped isometric dorsiflexions were performed during testing. Both contractions included a ramp up phase to 10% MVC and ramp down phases from 10% MVC that were performed at 10% MVC/s. However, the sombrero-shaped dorsiflexion had an additional 10 s ramp up phase to 30% MVC and 10 s ramp down phase back to 10% MVC. For sombrero contractions, MUs recruited prior to the superimposed triangle were labelled brim units and additionally recruited units from the superimposed triangle onwards were labelled cap units. Two plateaus of steady-state forces could be examined from each contraction type (P1 and P2). C, maximal and submaximal dorsiflexions were performed pre- and post-drug ingestion. The contraction protocol consisted of submaximal trapezoidal dorsiflexions to 10%, 20% and 30% MVC, pre-drug sombreros, post-drug trapezoidal dorsiflexions to 10% of MVC and post-drug sombreros. Submaximal trapezoids were used to assist decomposition and tracking of MUs by calibrating MU filters.

and secured with a non-compliant, ratchet type binding to a custom designed foot plate which incorporated a commercially available torque sensor (capacity = 565 Nm, Model 2110–5K; Honeywell International Inc., Charlotte, NC, USA). The foot plate was mounted on an aluminium frame which was secured to the therapy chair, with the torque sensor axis of rotation aligned to participants' malleoli. Isometric ankle torque was sampled at 2000 Hz using a Power 1401 interface with Spike2 software (version 7, Cambridge Electronic Design Ltd., UK). Feedback for the unfiltered torque signal was displayed on a computer monitor positioned ~1 m in front of the participant, with dorsiflexion torque presented as a positive inflection in torque on the screen.

Experimental protocol

Maximal voluntary contraction (MVC). Dorsiflexion MVC was determined before and after the cyproheptadine intervention (Fig. 1C). Care was taken during all maximal contractions to ensure that participants minimised the use of muscles other than the tibialis anterior to generate isometric dorsiflexion torque. An investigator provided instructions and a demonstration of the task prior to participants attempting the task. sEMG activity from antagonistic muscles was constantly monitored throughout the session, and any trial with excessive activity from antagonist muscles was deemed a mistrial and repeated. After familiarisation, participants performed five maximal effort dorsiflexions of ~3 s in duration with rest periods of up to 3 min between contractions. The trial that generated the highest magnitude of torque was determined to be the participant's MVC. This value was then used to set the submaximal dorsiflexion targets for each participant.

Contraction protocol. After dorsiflexion MVC was established, the participants performed submaximal trapezoidal contractions to 10%, 20% and 30% of MVC (Fig. 1C). Each trapezoid had a rate of torque development and decline of 10% MVC/s. The 10% MVC trapezoid plateau lasted 40 s so that its duration was consistent with the duration of the sombrero trials (Fig. 1B). The 20% and 30% MVC trapezoid plateaus lasted 10 s. These trapezoidal contractions served as calibration trials to prime the MU separation filters during decomposition (see section 'HDsEMG analysis'). Following this, participants then performed five repetitions of pre-drug sombrero contractions. The sombrero contraction has been previously used during ankle dorsiflexions (Beauchamp et al., 2023) and is comprised of two 10 s plateaus at 10% MVC, separated by a 20 s superimposed triangular contraction which peaked at 30% MVC (Fig. 1B). Following the drug intervention,

participants completed additional dorsiflexion MVCs, 10% MVC trapezoids and sombrero contractions.

Electromyography

Muscle activity for the tibialis anterior was measured using a semi-disposable 64-channel HDsEMG grid electrode (8 × 8) with a 10 mm inter-electrode distance (OTBIOelettronica, Torino, Italy). Following skin preparation (shaving, abrasion and cleansing with 70% isopropyl alcohol), the position and orientation of the electrode grid was determined by an experienced investigator via palpation of the right tibialis anterior muscle belly. Electrodes were fixed to the middle of the muscle belly using a bi-adhesive, perforated foam layer and conductive paste (SpesMedica, Battipaglia, Italy). A dampened strap ground electrode (OTBIOelettronica, Torino, Italy) was positioned over the right ankle malleoli. HDsEMG signals were recorded in monopolar mode and converted to digital signal by a 16-bit wireless amplifier (Sessantaquattro, OTBIOelettronica, Torino, Italy). HDsEMG signals were recorded and visualised using OTBioLab+ software (version 1.3.0., OTBIOelettronica, Torino, Italy). Additionally, bipolar surface EMG (sEMG) electrodes were attached to the plantarflexor muscles of the test leg. Specifically, 24 mm Ag/AgCl electrodes (Kendall ARBO; Cardinal Health, Dublin OH, USA) were placed over the medial gastrocnemius and soleus. Electrodes were aligned parallel to the underlying muscles fibres, with an inter-electrode distance of 24 mm. EMG signals were differentially amplified (×1000) by a NL844 pre-amplifier, and bandpass filtered (10–500 Hz) by a NL135 Low Pass Filter and NL144 High Pass Filter (Digitimer Ltd., UK). Surface EMG was sampled at 2000 Hz via a Power 1401 interface with Spike2 software (version 7, Cambridge Electronic Design Ltd., UK).

HDsEMG analysis

Prior to decomposition, monopolar HDsEMG signals were digitally band pass filtered at 20–500 Hz with a second-order Butterworth filter. HDsEMG signals were decomposed into individual MU action potentials using blind source separation, via the convolutive kernel compensation method (Holobar & Zazula, 2007). This method has been validated previously for a broad range of contraction intensities of the tibialis anterior muscle (Del Vecchio, Casolo, et al., 2019; Del Vecchio, Negro, et al., 2019; Holobar et al., 2014; Negro et al., 2016). Blind source separation via convolution kernel compensation separates EMG activity into individual MU components by detecting the unique action potential waveform shapes, temporal and frequency characteristics (Holobar & Zazula, 2007; Holobar et al., 2014). This

process assumes the stationarity of the characteristics, an assumption which is challenged during the superimposed triangle component of the sombrero contraction. To overcome the likely challenges associated with identifying MUs during the sombrero contractions, a series of calibration trapezoidal contractions were concatenated to sombrero trials. Specifically, trapezoids with a steady state lasting ≥ 10 s at 10%, 20% and 30% of MVC were used to improve the yield of MUs from sombrero trials. The decomposition accuracy was assessed using pulse-to-noise ratio dB during each individual contraction (Holobar et al., 2014), and decomposed spike trains showing pulse-to-noise ratios < 30 dB were discarded from the analysis (Del Vecchio et al. (2020)). All MU pulse trains were manually inspected by an investigator experienced in MU analysis, and only pulse trains with a reliable discharge pattern were considered for tracking and analysis.

Motor unit tracking

MUs were tracked in the current study via the decomposition filter method, which has previously demonstrated high reliability during pharmacological interventions (Goodlich et al., 2023b). To investigate the effect of contraction type, MUs were tracked between the pre-drug 10% MVC trapezoid and pre-drug sombrero contractions. To investigate the effect of drug, MUs were tracked from pre-drug to post-drug sombreros. All tracked MU spike trains were divided in time-based epochs, where plateau 1 was defined as the middle 6 s of the first 10 s hold at 10% of MVC, and plateau 2 was defined as the middle 6 s of the second 10 s hold at 10% of MVC. The middle 6 s was used to ensure the steady state of each hold was captured.

Motor unit spike train analysis

Average discharge rate, spike count and spike variability were extracted from each epoch. MU spike count represents the number of firings detected during the steady state of each plateau phase and is dependent on the duration of MU firing. Discharge rate was calculated as the average rate at which an MU fires within the plateau, measured in pulses per second (pps), and is independent of firing duration. MU spike variability was calculated as the coefficient of variation of the interspike intervals (CoV ISI) during each plateau phase. Additionally, MU recruitment threshold and derecruitment threshold were calculated as the torque value corresponding to the first and last MU firing, respectively. Peak discharge rate determined as the highest instantaneous discharge rate value for each MU identified during sombrero contractions. To quantify self-sustained firing behaviour before

and after the drug intervention, firing duration was calculated as the time in seconds that a MU fired for from peak force (i.e. mid sombrero) until its eventual derecruitment.

Statistical analysis

All statistical analysis was performed in R, using RStudio (version 4.1.1; R Foundation for Statistical Computing, Vienna, Austria). Linear mixed effects models were used for MU analysis as they allow for the inclusion of all units, whilst accounting for the hierarchical nature of the data (i.e. higher correlation for units within subjects compared with between subjects) (Tenan et al., 2014; Yu et al., 2021). Models were developed by iteratively adding predictor variables or interaction effects, and the fit of models was compared using an ANOVA. Separate linear mixed effects models were developed using the *nlme* package (Pinheiro et al., 2017) to evaluate the effect of contraction type (i.e. trapezoidal and sombrero) and drug (i.e. pre- and post-cyproheptadine) on each outcome measure of interest. Within the plateau regions, the outcome measures of interest were MU discharge rate, spike count and spike variability. Additional outcome measures include MU recruitment threshold, derecruitment threshold and firing duration. The first set of models considered a fixed effect of contraction type (i.e. trapezoidal and sombrero) and fixed effect of plateau, as well as the interaction effect between contraction and plateau, with a random intercept for each subject, and MUs nested within subjects (e.g. Discharge rate \sim (Plateau * Contraction) + (1 | subject ID) + (1 | subject ID: MU ID)). The second set of models considered a fixed effect of drug (i.e. pre-drug sombrero and post-drug sombrero) and fixed effect of plateau, as well as the interaction effect between drug and plateau, with a random intercept for each subject, and MUs nested within subjects (e.g. Spike count \sim (Plateau * Drug) + (1 | subject ID) + (1 | subject ID: MU ID)). Significance was calculated using the *lmerTest* package in R (Kuznetsova et al. 2017), which utilises Satterthwaite's method to approximate degrees of freedom and generate *P* values for mixed effect models by comparing the full model (with the effect of interest) against a null model (excluding the effect of interest). In the event of a significant fixed or interaction effect, pairwise *post hoc* tests were conducted to examine estimated marginal means (EMM) with 95% confidence intervals using the *emmeans* package (Lenth & Lenth, 2018). The Kenward–Roger approximation was used for estimating degrees of freedom for the *post hoc* pairwise comparisons, and the Tukey method for comparing estimates was used to adjust *P* values and confidence levels. Cohen's *d* effect sizes were calculated for data from tracked MUs to estimate the magnitude of change for significant *post hoc* comparisons. For all

Table 1. Anthropometric and maximal isometric dorsiflexion torque

Age, years	24.9 ± 2.8
Height, m	1.78 ± 0.1
Mass, kg	78.4 ± 13.7
Body mass index, kg/m ²	24.7 ± 3.1
Dorsiflexion MVC, Nm	
Pre-drug	58.0 ± 17.9
Post-drug	57.2 ± 18.1

MVC are presented as group means ± SD (*n* = 12, 5 female). MVC, maximal voluntary contraction; sEMG, surface electromyography; RMS, root mean square; mV, millivolts.

statistical comparisons, an α value of $P < 0.05$ was considered statistically significant.

Results

Participant characteristics and motor unit decomposition

The age and anthropometric characteristics of participants are described in Table 1. Two individuals (one male and one female) were excluded from statistical analysis due to an inability to reliably identify and track MUs across all contractions. There was no significant difference in maximal dorsiflexion torque between pre- and post-cyproheptadine ($F_{1,10} = 1.21$, $P = 0.2962$). Decomposition of HDsEMG signals yielded total of 233 MU spike trains. The average number of MUs tracked from trapezoidal to sombrero-shaped contractions was 19 ± 10 per subject. The average number of MUs tracked from pre- to post-drug during sombrero-shaped contractions was 18 ± 11 per subject.

Motor unit characteristics during trapezoid- and sombrero-shaped contractions

An instantaneous discharge rate (IDR) of a representative MU that was tracked between a trapezoid and sombrero-shaped contraction is presented in Fig. 2A. When performing the same relative force there was consistency in MU discharge profiles between plateau 1 and plateau 2 of the 10% MVC trapezoidal contraction. However, when the same 10% MVC force was performed with an additional period of increased voluntary drive in the middle of the contraction (i.e. a sombrero contraction), there was a notable reduction in MU discharge profiles from plateau 1 to plateau 2. There were two clear profiles of MU activation during sombrero-shaped contractions. One profile was associated

with spike trains for the 'brim' part of the sombrero and the other profile was associated with spike trains for the 'cap' part of the sombrero. Of particular interest are units which are recruited into the cap phase of the sombrero which show prolonged firing after the superimposed descending drive ends (i.e. plateau 2).

A contraction by plateau interaction effect ($F_{1,259.69} = 29.39$, $P < 0.0001$, Fig. 2B), was identified for MU spike count. Spike count was significantly lower for plateau 2 than plateau 1 for the sombrero contraction ($P < 0.0001$, $d = 0.75$ [0.49, 1.09], difference in EMM = 21.36 spikes [13.15, 29.58]), which was reflected by the rightward shift in distribution of spike count change scores from plateau 1 to plateau 2 (Fig. 2E). Compared with the trapezoidal contraction, MU spike count was also significantly lower for the sombrero contraction for plateau 1 ($P < 0.0001$, $d = 0.72$ [0.43, 1.11], difference in EMM = 18.35 spikes [9.76, 26.95]) and plateau 2 ($P < 0.0001$, $d = 1.38$ [1.19, 1.63], difference in EMM = 41.76 spikes [34.19, 49.34]).

Similar to MU spike count, a contraction by plateau interaction was identified for MU discharge rate ($F_{1,249.93} = 82.00$, $P < 0.0001$, Fig. 2C), where discharge rate was significantly lower for plateau 2 than plateau 1 for the trapezoid ($P = 0.0053$, $d = 0.33$ [0.09, 0.64], difference in EMM = 0.45 pps [0.10, 0.79]) and the sombrero ($P < 0.0001$, $d = 1.98$ [1.65, 2.52], difference in EMM = 2.24 pps [1.86, 2.62]) contractions. This is reflected by the rightward shift in the distribution of plateau 1 to plateau 2 change scores for trapezoidal contractions, and further rightward shift for sombrero contractions (Fig. 2F). Although the MU discharge rate decreased from the first to second plateau for both types of contraction, the discharge rate was even lower for sombrero contractions at plateau 2 than trapezoid contractions at plateau 2 ($P < 0.0001$, $d = 0.39$ [0.24, 0.52], difference in EMM = 2.02 pps [1.67, 2.37]).

For MU spike variability, an interaction effect between contraction type and plateau was identified ($F_{1,226.92} = 17.56$, $P < 0.0001$, Fig. 2D). MU variability was significantly lower for plateau 2 than plateau 1 for the trapezoid ($P = 0.0118$, $d = 0.17$ [-0.04, 0.37], difference in EMM = 0.020 [0.003, 0.037]), but significantly higher for plateau 2 than plateau 1 for the sombrero ($P < 0.0186$, $d = -0.43$ [-0.69, -0.20], difference in EMM = -0.027 [-0.052, -0.003]) contractions. The distribution of plateau 1 to plateau 2 spike variability change scores demonstrated a rightward shift during the trapezoidal contractions and leftward shift during the sombrero contractions (Fig. 2G). Compared with the trapezoidal contraction, spike variability was significantly greater for the sombrero contraction for plateau 2 ($P < 0.0001$, $d = -0.68$ [-1.02, -0.50], difference in EMM = -0.044 [-0.066, -0.021]).

5-HT₂ antagonism and motor unit characteristics during sombrero-shaped contractions

All participants were able to successfully perform sombrero-shaped contractions pre- and post-drug. Representative pre-drug spike trains are presented in Fig. 3A, where self-sustained firing was evident following the cap part of the sombrero. Interestingly, MUs that were detected in the cap prior to the drug intervention were recruited earlier with 5-HT₂ antagonism to perform the same 10% MVC contraction (i.e. plateau 1, see yellow and light green units in Fig. 3A). Furthermore, self-sustained firing of MUs that were recruited during the cap were abolished, or reduced in duration, with 5-HT₂ receptor antagonism (i.e. see green and light blue units that

discharge in plateau 2 pre-drug, but not post-drug, in Fig. 3A). Profiles of IDR for tracked MUs suggested that a suppression in firing occurred with 5-HT₂ antagonism which was evident for both plateaus, and peak discharge, for the sombrero contraction (Fig. 3B).

Drug effects on MUs recruited during cap forces

MUs that were identified for pre-drug during the cap phase of force generation were tracked across the cap phase for post-drug contractions. A drug by plateau interaction effect was identified for MU spike count ($F_{1,360} = 32.68$, $P < 0.0001$, Fig. 4B). Spike count was significantly lower in the pre-drug condition during

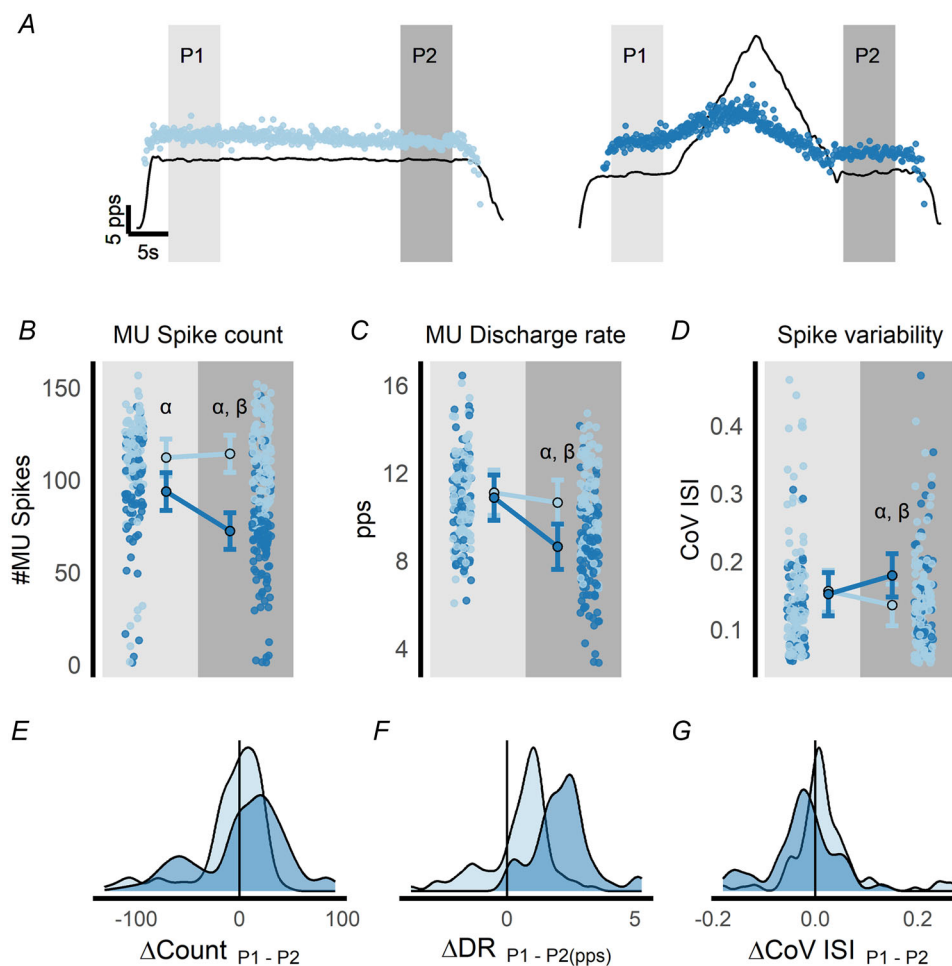


Figure 2. Motor unit discharge characteristics during trapezoid- and sombrero-shaped contractions
Light blue data reflect MU characteristics during trapezoid contractions whereas dark blue data reflect MU characteristics during sombrero contractions. Light grey area indicates plateau 1 (P1), and dark grey area indicates plateau 2 (P2). A, instantaneous discharge rate for same MU tracked between contraction types. B–D, MU spike count, discharge rate and spike variability. Each symbol represents data of an individual MU. The continuous line represents the group mean. E–G, change score distributions for MU spike count, discharge rate and spike variability, where each distribution represents the density of change scores from plateau 1 to plateau 2. α = *post hoc* significant contraction effect, $P < 0.05$. β = *post hoc* significant plateau effect, $P < 0.05$. MU, motor unit; pps, pulses per second; CoV, coefficient of variation; ISI, inter-spike interval; DR, discharge rate.

plateau 1 ($P < 0.0001$, $d = -1.01$ [$-1.66, -0.65$], difference in EMM = -53.81 spikes [$-73.47, -34.10$]), which was a factor of cap MUs being inactive during the pre-drug contractions but recruited earlier with 5-HT₂ antagonism. In contrast, spike count for MUs pre-drug was significantly higher than post-drug measurements for plateau 2 ($P = 0.003$, $d = 1.05$ [$0.77, 1.56$], difference in EMM = 26.91 spikes [$7.25, 46.60$]) which was a factor of spike count significantly increasing from plateau 1 to plateau 2 during the pre-drug condition ($P < 0.0001$, $d = -2.39$ [$-3.52, -1.86$], difference in EMM = -62.06 spikes [$-79.58, -44.50$]). Thus, 5-HT₂ antagonism blunted the normal physiological MU responses that was observed during the contraction.

By definition, cap units delineate from brim units because, under physiological conditions, they do not spike in plateau 1. Therefore, there is no model estimate for pre-drug plateau 1 discharge rate or spike variability values as there were no spikes from which to calculate these metrics. Fixed effects of drug ($F_{1,347.33} = 13.68$,

$P = 0.0003$) and plateau ($F_{1,347.33} = 13.68$, $P = 0.0003$) were identified for MU discharge rate (Fig. 4C). *Post hoc* testing revealed that post-drug discharge rate reduces from plateau 1 to plateau 2 ($P < 0.0001$, $d = 2.19$ [$1.36, 4.8$], difference in EMM = 3.19 pps [$2.21, 4.16$]) and pre-drug plateau 2 discharge rate was significantly higher than post-drug values ($P = 0.0009$, $d = 1.25$ [$0.80, 2.17$], difference in EMM = 1.26 pps [$0.46, 2.05$]). For MU spike variability (Fig. 4D), no fixed effects of drug ($F_{1,22.43} = 1.75$, $P = 0.1986$) or plateau ($F_{1,23.61} = 1.57$, $P = 0.2222$) were identified.

Drug effects on motor units actively firing during both brim regions

Identified MUs that were firing action potentials during both brim phases of force generation pre-drug were tracked across the brim phases for post-drug contractions. A fixed effect of drug ($F_{1,163.6} = 4.86$, $P = 0.02892$) and plateau ($F_{1,156.33} = 38.50$, $P < 0.0001$) was identified for MU spike count (Fig. 4F), whereby post-drug spike count was lower than pre-drug ($d = 0.27$ [$0.09, 0.42$], difference in EMM = 7.45 spikes [$0.76, 14.10$]) and plateau 2 spike count was lower than plateau 1 ($d = 0.63$ [$0.37, 0.97$], difference in EMM = 20.6 spikes [$14.1, 27.2$]). A drug by plateau interaction effect was identified for MU discharge rate ($F_{1,150.8} = 6.92$, $P = 0.0094$). *Post hoc* testing revealed that post-drug discharge rate was lower than pre-drug values at plateau 2 ($P = 0.0001$, $d = 0.61$ [$0.37, 0.95$], difference in EMM = 0.89 pps [$0.37, 1.41$]), but not plateau 1 ($P = 0.8912$). MU discharge rate during plateau 2 was significantly lower than plateau 1 for both pre-drug ($P < 0.0001$, $d = 2.0$ [$1.62, 2.70$], difference in EMM = 2.04 pps [$1.54, 2.53$]) and post-drug values ($P < 0.0001$, $d = 2.09$ [$1.55, 2.94$], difference in EMM = 2.78 pps [$2.23, 3.32$]). A fixed effect of plateau was identified for MU spike variability ($F_{1,143.59} = 7.30$, $P = 0.0077$, Fig. 4H), whereby spike variability increased from plateau 1 to plateau 2 ($d = -0.1$ [$-0.41, 0.09$], difference in EMM = -0.02 [$-0.03, -0.01$]). No fixed effect of drug was detected for MU spike variability ($F_{1,146.45} = 3.47$, $P = 0.0647$).

Drug effects on recruitment threshold, peak discharge rate and derecruitment threshold

A subtle but significant difference in MU recruitment threshold was identified, whereby the recruitment threshold was lower after the ingestion of cyproheptadine ($F_{1,159.6} = 11.014$, $P = 0.00112$, estimated mean difference = 1.09% MVC [$0.44, 1.74$], $d = 0.24$ [$0.08, 0.42$]). Antagonism of 5-HT₂ receptors did not affect MU derecruitment threshold ($F_{1,158.81} = 0.576$, $P = 0.449$). Peak MU discharge rate was significantly lower after

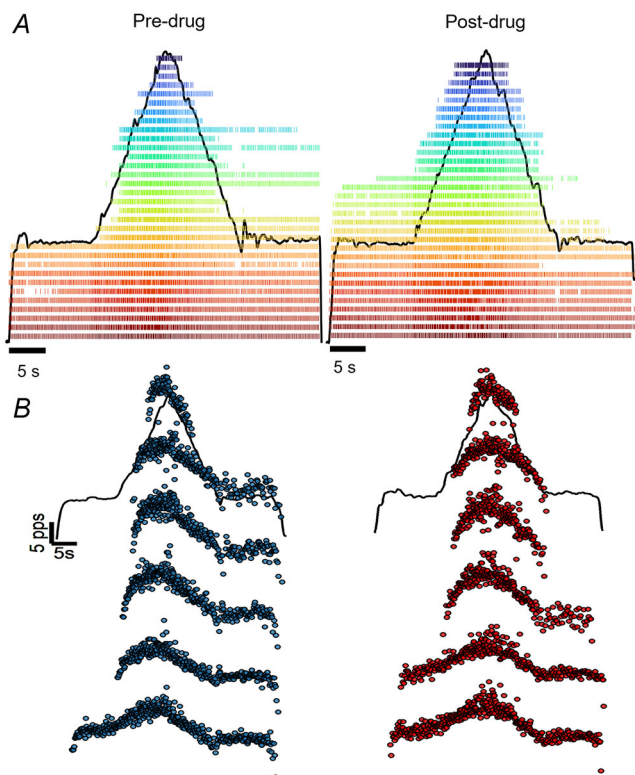


Figure 3. Sombrero-shaped contractions pre- and post-5-HT₂ antagonism

A, representative raster plots of MU spike trains identified from HDsEMG of one subject (age: 27, male). MUs were tracked between the drug condition, and colours in the raster plot indicate when the same MU was active during each contraction. B, instantaneous discharge rate of six MUs from the same subject. MUs were tracked from the pre-drug contraction (blue symbols) to the post-drug contraction (red symbols).

the ingestion of cyproheptadine ($F_{1,171.97} = 18.423$, $P < 0.001$, estimated mean difference = 1.65 pps [0.68, 2.62], $d = 0.34$ [0.19, 0.50]).

5-HT₂ antagonism and firing duration of MUs

The duration of MU firing was calculated from peak force during the contraction to the derecruitment of the MU. Both cap and brim unit firing duration were significantly lower post-drug (cap: $F_{1,107.2} = 20.50$, $P < 0.0001$, $d = 0.46$

[0.34, 0.59], difference in EMM = 1.77s [0.99, 2.54]; brim: $F_{1,56.99} = 4.50$, $P = 0.0382$, $d = 0.27$ [0.07, 0.43], difference in EMM = 0.88s [0.05, 1.71]; Fig. 5). It is notable that cap MU firing duration data forms clusters in two groups: those that fire for greater than 15 s, and those that fire for less than 10 s. Given that the contraction task ceased 20 s after peak force, many cap units continued to discharge in the 10 s period following peak force, as well as the 10 s after the superimposed descending drive finished (i.e. plateau 2). Nonetheless, self-sustained

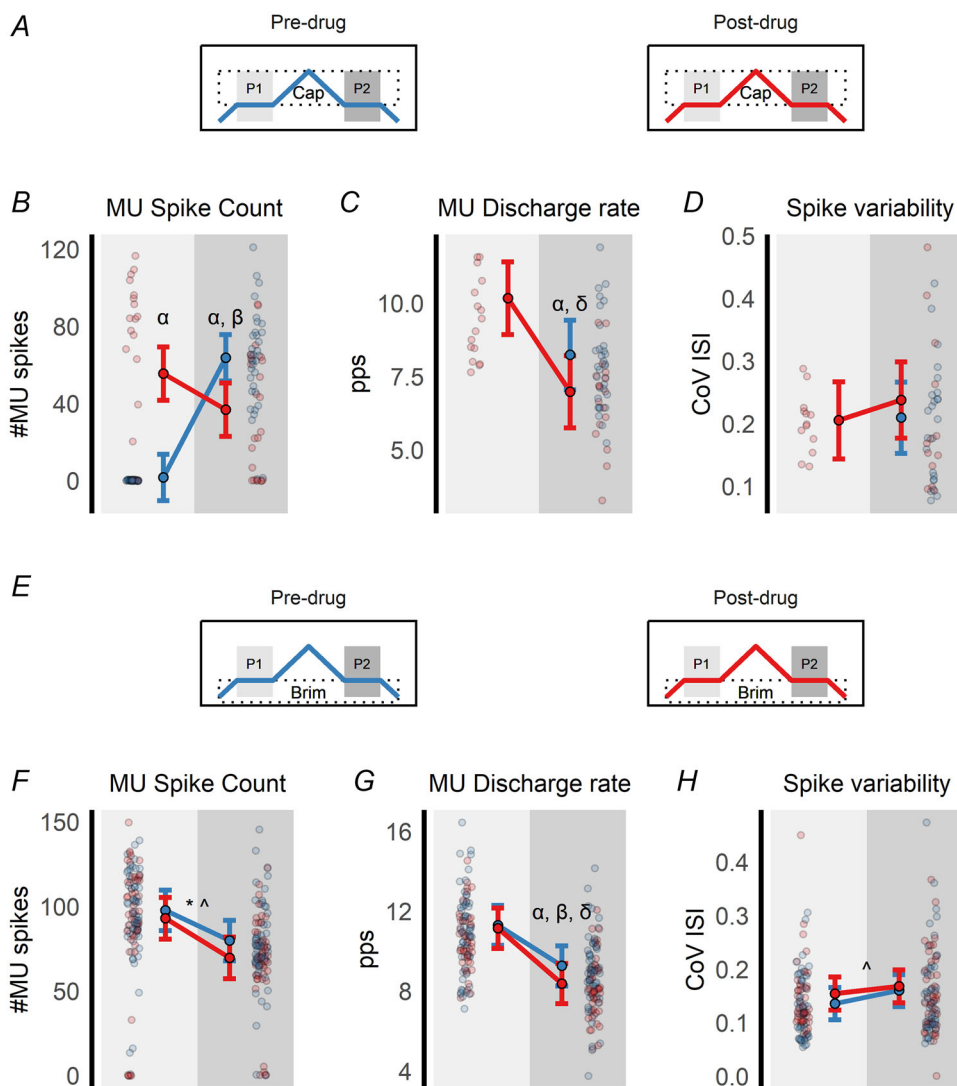


Figure 4. Motor unit firing characteristics for sombrero contractions with, and without, 5-HT₂ receptor antagonism

MU spike count, discharge rate, and spike variability of cap units (B–D) and brim units (F–H) are presented for MUs that were tracked pre- and post-drug intervention. Schematics are provided to differentiate cap units (A) and brim units (E). Pre-drug data are presented in blue and drug data are presented in red. Solid circles are estimated marginal means with error bars that indicate 95% confidence intervals. Transparent circles represent data for an individual MU. α = *post hoc*, significant drug effect, $P < 0.05$. β = *post hoc*, significant plateau effect during the pre-drug condition, $P < 0.05$. δ = *post hoc*, significant plateau effect during the post-drug condition, $P < 0.05$. * = fixed effect of drug, $P < 0.05$. ^ = fixed effect of plateau, $P < 0.05$. MU, motor unit; pps, pulses per second; CoV, coefficient of variation; ISI, inter-spike interval; DR, discharge rate; P1, plateau 1; P2, plateau 2.

firing was significantly reduced with 5-HT₂ antagonism regardless of firing duration.

Firing duration was also assessed across the whole contraction. Both cap and brim unit firing duration were significantly lower post-drug (cap: $F_{1,103.5} = 26.99$, $P < 0.0001$, $d = 0.54$ [0.42, 0.65], difference in EMM = 2.12 s [1.31, 2.92]; brim: $F_{1,54.27} = 7.36$, $P = 0.0089$, $d = 0.37$ [0.21, 0.54], difference in EMM = 1.23 s [0.32, 2.14]). Similar to firing duration calculated from peak force, there were larger drug effects on whole contraction firing duration for cap units compared with brim units.

Discussion

The purpose of this study was to examine how serotonergic neuromodulation contributes to self-sustained firing of motoneurons in humans. Our experiments showed that adding (and then removing) descending drive in the middle of steady-state contractions markedly suppresses discharge of active MUs and revealed that some newly recruited units in the superimposed phase were unable to switch off due to their self-sustained firing. Subsequently, a 5-HT₂ receptor antagonist was used to determine whether the changes in discharge rate and self-sustained firing were mediated by a serotonergic mechanism. The drug effects identified in this study indicated that (1) motoneurone discharge rate and self-sustained firing is suppressed with 5-HT₂ antagonism, and (2) reducing 5-HT₂ receptor

activity facilitates the recruitment of additional MUs to generate the same level of force that was generated in pre-drug conditions. Overall, this study provides novel evidence that serotonergic neuromodulation plays a key role in self-sustained firing behaviour of human motoneurons after the removal of descending drive. This study also provides evidence that reducing serotonergic neuromodulation in the human motor system leads to adaptative changes in MU recruitment strategies to meet the task demands.

Self-sustained firing is observable following superimposed descending drive

After the removal of synaptic current, the extra depolarising current caused by PICs can facilitate self-sustained firing of the motoneurone. This phenomenon has been extensively studied in reduced preparations (Crone et al., 1988; Hounsgaard et al., 1988; Lee & Heckman, 1998b) and in humans using EMG techniques (Gorassini et al., 1998, 2002; Hassan et al., 2021; Kiehn & Eken, 1997; Mesquita et al., 2022; Orssatto et al., 2022; Vandenberk & Kalmar, 2014). The key feature of human experiments is the inclusion of linear and symmetrical isometric muscle contractions, where MU recruitment and derecruitment can be compared for symmetry when MUs are extracted from the EMG signal of the contracting muscle. We have previously detailed MU discharge and recruitment properties using trapezoidal-shaped contractions generated by ankle dorsiflexors (Goodlich et al., 2023a), where subtle signs of hysteretic behaviour are observable for contraction intensities up to 30% MVC. In the current study we reinforce that self-sustained firing in motoneurons is more readily observable during the performance of a 10% MVC trapezoidal-shaped contraction that includes a brief period where additional descending drive was delivered to the motoneurone pool (Beauchamp et al., 2023). Additional descending drive will necessarily recruit higher threshold MUs (Adrian & Bronk, 1929; Henneman, 1977) and the linear ramp will cause PIC activation for these MUs (Binder et al., 2020). Given that deactivation of PICs requires an additional source of synaptic inhibition, or complete cessation of excitatory inputs to the motoneurone, the self-sustained firing that occurred after the removal of additional descending drive was a product of excitation outweighing inhibition. It is possible that the excitatory drive required to maintain the steady-state contraction (i.e. the lower threshold MUs) provided a weak source of excitation for higher threshold MUs recruited during the cap phase of the sombrero contraction. Indeed, a known role of PICs is to amplify the effects of ionotropic inputs to motoneurons, where an

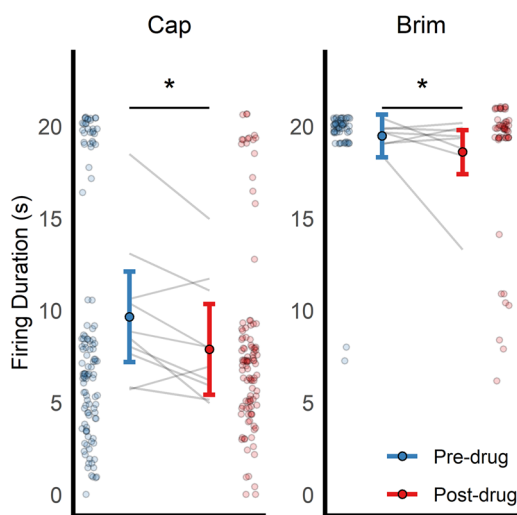


Figure 5. Firing duration of motor units identified for sombrero contractions

Each transparent circle represents an individual MU which has been tracked between pre-drug (blue) and post-drug (red) contractions. Grey lines indicate subject averages for MU firing duration. The solid points represent the estimated marginal mean, with 95% confidence intervals indicated with error bars. * = fixed effect of drug, $P < 0.05$.

active PIC can cause depolarisation of the motoneurone at lower levels of excitatory synaptic input.

Motor unit discharge and spike count are reduced after superimposed descending drive

Despite similar MU firing properties in the first plateau for each contraction task, MUs that were tracked throughout the experiment had reductions in discharge rate and spike count, as well as an increase in spiking variability, during the second plateau of the sombrero-shaped contraction. Therefore, delivering additional descending drive to motoneurons during a sustained contraction reduces the output of the motoneurone with more time-variant generation of MU action potentials. The magnitude of differences in MU discharge between the trapezoid and sombrero contraction may suggest that multiple mechanisms contributed to changes in MU firing. In particular, the additional superimposed descending drive in the middle of the steady-state contraction caused MU discharge to reduce by 2.24 pps, which is a fivefold exacerbation of effects compared with the regular steady-state contraction (reduction of only 0.45 pps). A point of difference between the contraction protocols was that additional MUs were recruited during the cap phase of the sombrero. As these MUs remained firing when descending drive was removed, the additional force generated by the self-sustained firing MUs would necessitate a reduction in MU discharge from the brim units to generate the same 10% MVC force from plateau 1 to plateau 2. To date, it is unknown whether serotonergic neuromodulation substantially contributes to such changes in MU behaviour.

Motor unit discharge rate and self-sustained firing is suppressed with 5-HT₂ antagonism

In the current study, a 5-HT₂ receptor antagonist was used to determine the influence that 5-HT has on human motoneurone properties by identifying how 5-HT₂ activity affects MUs during, and following, the addition of superimposed descending drive on a steady-state contraction. 5-HT₂ receptor antagonism not only reduced discharge rate during the cap and brim components of the sombrero-shaped contractions, but also reduced the incidence and duration of self-sustained firing of the cap units. Therefore, the serotonergic component of the neuromodulatory system contributed to shaping the behaviour of MU firing during voluntary contractions in humans, which is mediated by 5-HT₂ receptor activity in the CNS.

In humans, antagonism of the 5-HT₂ receptor has been associated with a reduction in rate of torque development (Goodlich et al., 2022), MU discharge rate (Goodlich

et al., 2022, 2023a) and reductions in Delta F, which is the most common method of estimating PIC magnitude in humans (D'Amico et al., 2013; Goodlich et al., 2023a). The reduction in Delta F suggests that 5-HT₂ plays a role in MU firing rate hysteresis, which is supported in the current study whereby 5-HT₂ antagonism reduced self-sustained firing to cause a more symmetrical firing rate profile. 5-HT can promote depolarisation of the motoneurone via a number of mechanisms, including facilitation of rectifying inward currents (Hsiao et al., 1997; Takahashi & Berger, 1990), facilitation of low voltage Ca²⁺ currents (Berger & Takahashi, 1990), and inhibition of K⁺ leak conductance (Elliott & Wallis, 1992; Perrier et al., 2003). Alternatively, motoneurone firing can also be enhanced by modulating afterhyperpolarization (AHP), as 5-HT reduces K⁺ currents responsible for sAHP (Hounsgaard & Kiehn, 1989) and mAHP (Grunnet et al., 2004) phases of the action potential.

The hallmark of PIC induced self-sustained firing is discharge hysteresis, whereby motoneurone derecruitment occurs at a lower level of excitatory input than was required to initially recruit the motoneurone (Bennett et al., 1998; Hounsgaard & Kiehn, 1989; Lee & Heckman, 1996). Animal and cellular preparations have clearly demonstrated that 5-HT innervation of motoneurons can induce self-sustained firing (Hounsgaard & Kiehn, 1985; Hounsgaard et al., 1988). Notably, self-sustained firing behaviour disappears after an acute spinal transection in cat models, where descending projections from the raphe–spinal pathways are interrupted and cannot access the motoneurone pool (Hounsgaard et al., 1988). However, self-sustained firing is able to re-emerge following intravenous injection of the 5-HT precursor, 5-hydroxytryptophan, which provides a link between CNS 5-HT availability and self-sustained firing behaviour (Hounsgaard et al., 1988). In the current study, antagonism of the 5-HT₂ receptor reduced firing durations associated with MUs recruited in the brim, but the durations of MUs recruited in the cap of the sombrero-shaped contraction were reduced to a greater extent. Therefore, the current study provides evidence that the serotonergic system contributes to self-sustained firing of MUs of the tibialis anterior in humans.

5-HT activates G-protein coupled receptors which facilitate voltage sensitive ion channels on the dendrites of motoneurons (Lipscombe et al., 2004; Ma et al., 1997; Mantegazza et al., 2005). Therefore, competitive antagonism of the excitatory effects of 5-HT on the motoneurone likely constrained the PIC from perpetuating firing in the current study. Thus, for MUs that were only sustaining firing because of their PIC (and not excitatory inputs) the removal of 5-HT₂ activity caused attenuation of firing. Given that higher threshold units derecruit before lower threshold units, and participants were still activating the tibialis anterior

motor pool to produce force (10% of MVC), attenuation of firing was most observable for the cap MUs. It is likely that the lower threshold brim MUs maintained firing via excitatory synaptic input from descending sources and were therefore less affected by 5-HT₂ antagonism. Notably, some cap units did not show evidence of self-sustained firing after the descending drive was removed. Given that cessation of self-sustained is closely aligned with inhibitory inputs to the motor pool (i.e. reciprocal inhibition), it is quite likely that some, but not all, motoneurons were influenced by a form of inhibition when participants were attempting the down ramp of the cap. However, it is also possible that Ca²⁺ and Na⁺ mechanisms that form the basis of PICs contributed to the absence of self-sustained firing in some motoneurons. Na⁺ PICs are crucial for the initiation of repetitive discharge of motoneurons (Harvey et al., 2006; Kuo et al., 2006), and as such there is still likely PIC activity underpinning activation in these units. However, if L-type voltage-gated Ca²⁺ channels are differentially expressed across motoneurons it may lead to some, but not all, motoneurons exhibiting self-sustained firing. Nonetheless, antagonism of the 5-HT₂ receptor had the overall effect of hindering the PIC, thus suppressing self-sustained firing of MUs.

5-HT₂ receptor antagonism modifies motor unit behaviour to meet task demands

With an overall reduction in MU firing rate, maintaining a prescribed level of force following 5-HT₂ receptor antagonism could only be achieved with changes in MU recruitment. The earlier recruitment of more units to contribute to the force output is necessitated by an overall reduction in firing rates of the motor pool, irrespective of when discharge rate is assessed (Fig. 4F). This was noticeable for MUs recruited in the cap phase of the sombrero, where recruitment occurred with additional descending drive before drug administration, and recruitment occurred prior to the additional descending drive with 5-HT₂ antagonism. Hence, MUs that were previously being recruited into the contraction during the superimposed phase were now being recruited earlier into the initial steady-state hold (i.e. plateau 1). Although earlier MU recruitment could suggest an increase in intrinsic motoneurone excitability, serotonin antagonism would most likely reduce intrinsic excitability causing later MU recruitment. Earlier MU recruitment in the current study may instead reflect greater ionotropic input to the motor pool during the post-drug condition. If there was an increase in ionotropic synaptic input in the post-drug condition, this would have also increased the instances and duration of self-sustained firing, thus attenuating the magnitude of drug effects observed in

the present study. Nonetheless, more work is required to unpack discrepancies in the levels and types of descending synaptic input following 5-HT₂ antagonism.

Identification of changes in MU recruitment as a strategy to maintain a prescribed level of force following 5-HT₂ receptor antagonism has implications for several investigations that have examined the role of the serotonergic system in generating motor activity. For example, there are several reports where MVC force is compromised with 5-HT₂ antagonism (Henderson et al., 2022; Thorstensen et al., 2021, 2022). If MUs discharge slower, have a lower peak discharge rate, and are recruited earlier into the contraction, there may be limitations on generating higher contraction forces. However, this assumes that the effects of PIC activity, 5-HT₂ activity, and 5-HT release onto motoneurons is ubiquitous across all MUs and contraction forces. At present, it is unknown how these factors interact in humans when strong voluntary contractions are performed. However, a body of evidence is developing that suggests excitatory drive is necessary to observe 5-HT effects on motoneurone excitability in humans, and these effects align with estimates of PIC activity and self-sustained firing for MUs recruited in contractions up to 30% MVC.

Considerations

It must be acknowledged that in addition to antagonism of the 5-HT₂ receptor, cyproheptadine also has anti-histaminergic and anticholinergic effects via the antagonism of the H and M receptors, respectively. In slice preparations of rat motoneurons, there is evidence that histamine directly depolarises motoneurons (Wu et al., 2012) and can modify locomotor behaviour via spinal circuits (Coslovich et al., 2018). Thus, the effects reported in the current experiment may, in part, be influenced by antagonism of H or M receptors. However, it is important to note that histaminergic and cholinergic effects on muscle activation in humans may be less functional than those identified in animal or cellular preparations of motoneurons. Although the effects of antihistamines have not been assessed in human MU investigations, a potent antihistaminergic and antimuscarinic drug (promethazine) generates almost no effects on cortico-spinal excitability or motoneurone excitability across a wide range of muscle contractions in humans (Dempsey & Kavanagh, 2021, 2023). Cyproheptadine has repeatedly been used for the antagonism of the serotonergic system both by our lab group (Goodlich et al., 2022, 2023a; Henderson et al., 2024; Thorstensen et al., 2021, 2022) and other groups (D'Amico et al., 2013; Murray et al., 2010, 2011; Wei et al., 2014). Although these studies could not separate antihistaminergic effects from antiserotonergic effects, almost every investigation

highlighted the close alignment between their human findings and animal/cellular preparations that have used targeted 5-HT drugs.

Conclusion

The present study provides new evidence that implicates the 5-HT₂ receptor in the modulation of human MU discharge properties, where antagonism of 5-HT₂ receptors with cyproheptadine globally reduces discharge rates, recruitment thresholds, and self-sustained firing duration. Collectively, these results support the viewpoint that serotonergic neuromodulation plays a key role in the self-sustained firing behaviour of human motoneurons during voluntary contractions. Furthermore, reducing the influence of serotonergic neuromodulation in the motor system leads to adaptative changes in MU recruitment to meet the task demands.

References

- Adrian, E. D., & Bronk, D. W. (1929). The discharge of impulses in motor nerve fibres: Part II. The frequency of discharge in reflex and voluntary contractions. *The Journal of Physiology*, **67**(2), 9–151.
- Beauchamp, J. A., Pearcey, G. E., Khurram, O. U., Negro, F., Dewald, J. P., & Heckman, C. (2023). Intrinsic properties of spinal motoneurons degrade ankle torque control in humans. *bioRxiv*. <https://doi.org/10.1101/2023.10.23.563670>
- Bennett, D. J., Hultborn, H., Fedirchuk, B., & Gorassini, M. (1998). Synaptic activation of plateaus in hindlimb motoneurons of decerebrate cats. *Journal of Neurophysiology*, **80**(4), 2023–2037.
- Berger, A. J., & Takahashi, T. (1990). Serotonin enhances a low-voltage-activated calcium current in rat spinal motoneurons. *Journal of Neuroscience*, **10**(6), 1922–1928.
- Binder, M. D., Powers, R. K., & Heckman, C. (2020). Non-linear input-output functions of motoneurons. *Physiology*, **35**(1), 31–39.
- Boess, F., & Martin, I. (1994). Molecular biology of 5-HT receptors. *Neuropharmacology*, **33**(3–4), 275–317.
- Coslovich, T., Brumley, M. R., D'angelo, G., Della Mora, A., Swann, H. E., Ortolani, F., & Taccola, G. (2018). Histamine modulates spinal motoneurons and locomotor circuits. *Journal of Neuroscience Research*, **96**(5), 889–900.
- Crone, C., Hultborn, H., Kiehn, O., Mazieres, L., & Wigström, H. (1988). Maintained changes in motoneuronal excitability by short-lasting synaptic inputs in the decerebrate cat. *The Journal of Physiology*, **405**(1), 321–343.
- D'Amico, J. M., Murray, K. C., Li, Y., Chan, K. M., Finlay, M. G., Bennett, D. J., & Gorassini, M. A. (2013). Constitutively active 5-HT₂/α1 receptors facilitate muscle spasms after human spinal cord injury. *Journal of Neurophysiology*, **109**(6), 1473–1484.
- Del Vecchio, A., Casolo, A., Negro, F., Scorcelletti, M., Bazzucchi, I., Enoka, R., Felici, F., & Farina, D. (2019). The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding. *The Journal of Physiology*, **597**(7), 1873–1887.
- Del Vecchio, A., Holobar, A., Falla, D., Felici, F., Enoka, R., & Farina, D. (2020). Tutorial: Analysis of motor unit discharge characteristics from high-density surface EMG signals. *Journal of Electromyography and Kinesiology*, 102426.
- Del Vecchio, A., Negro, F., Holobar, A., Casolo, A., Folland, J. P., Felici, F., & Farina, D. (2019). You are as fast as your motor neurons: Speed of recruitment and maximal discharge of motor neurons determine the maximal rate of force development in humans. *The Journal of Physiology*, **597**(9), 2445–2456.
- Dempsey, L. M., & Kavanagh, J. J. (2021). Muscarinic receptor blockade causes postcontraction enhancement in corticospinal excitability following maximal contractions. *Journal of Neurophysiology*, **125**(4), 1269–1278.
- Dempsey, L. M., & Kavanagh, J. J. (2023). Muscarinic acetylcholine activity modulates cortical silent period, but not motor evoked potentials, during muscle contractions. *Experimental Brain Research*, **241**(6), 1543–1553.
- Elliott, P., & Wallis, D. (1992). Serotonin and norepinephrine as mediators of altered excitability in neonatal rat motoneurons studied in vitro. *Neuroscience*, **47**(3), 533–544.
- Goodlich, B. I., Del Vecchio, A., Horan, S. A., & Kavanagh, J. J. (2023a). Blockade of 5-HT₂ receptors suppress motor unit firing and estimates of persistent inward currents during voluntary muscle contraction in humans. *The Journal of Physiology*, **601**(6), 1121–1138.
- Goodlich, B. I., Del Vecchio, A., & Kavanagh, J. J. (2023b). Motor unit tracking using blind source separation filters and waveform cross-correlations: Reliability under physiological and pharmacological conditions. *Journal of Applied Physiology*, **135**(2), 362–374.
- Goodlich, B. I., Horan, S. A., & Kavanagh, J. J. (2022). Blockade of 5-HT₂ receptors suppresses rate of torque development and motor unit discharge rate during rapid contractions. *Journal of Neurophysiology*, **127**(1), 150–160.
- Gorassini, M., Bennett, D. J., & Yang, J. F. (1998). Self-sustained firing of human motor units. *Neuroscience letters*, **247**(1), 13–16.
- Gorassini, M., Yang, J. F., Siu, M., & Bennett, D. J. (2002). Intrinsic activation of human motoneurons: Possible contribution to motor unit excitation. *Journal of Neurophysiology*, **87**(4), 1850–1858.
- Grunnet, M., Jespersen, T., & Perrier, J. F. (2004). 5-HT_{1A} receptors modulate small-conductance Ca²⁺-activated K⁺ channels. *Journal of Neuroscience Research*, **78**(6), 845–854.
- Harvey, P. J., Li, Y., Li, X., & Bennett, D. J. (2006). Persistent sodium currents and repetitive firing in motoneurons of the sacrocaudal spinal cord of adult rats. *Journal of Neurophysiology*, **96**(3), 1141–1157.
- Hassan, A., Fajardo, M. E., Cummings, M., McPherson, L. M., Negro, F., Dewald, J. P., Heckman, C. J., & Pearcey, G. E. (2021). Estimates of persistent inward currents are reduced in upper limb motor units of older adults. *The Journal of Physiology*, **599**(21), 4865–4882.

- Henderson, T. T., Taylor, J. L., Thorstensen, J. R., & Kavanagh, J. J. (2024). Excitatory drive to spinal motoneurons is necessary for serotonin to modulate motoneuron excitability via 5-HT₂ receptors in humans. *European Journal of Neuroscience*, **59**(1), 17–35.
- Henderson, T. T., Taylor, J. L., Thorstensen, J. R., Tucker, M. G., & Kavanagh, J. J. (2022). Enhanced availability of serotonin limits muscle activation during high-intensity, but not low-intensity, fatiguing contractions. *Journal of Neurophysiology*, **128**(4), 751–762.
- Henneman, E. (1977). Functional organization of motoneuron pools: The size principle. *International Union of Physiological Sciences*, **12**(50), 13–25.
- Holobar, A., Minetto, M. A., & Farina, D. (2014). Accurate identification of motor unit discharge patterns from high-density surface EMG and validation with a novel signal-based performance metric. *Journal of Neural Engineering*, **11**(1), 016008.
- Holobar, A., & Zazula, D. (2007). Multichannel blind source separation using convolution kernel compensation. *IEEE Transactions on Signal Processing*, **55**(9), 4487–4496.
- Honrubia, M. A., Rodriguez, J., Dominguez, R., Lozoya, E., Manaut, F., Seijas, J. A., Villaverde, M. C., Calleja, J. M., Cadavid, M. I., Maayani, S., Sanz, F., & Loza, M. I. (1997). Synthesis, affinity at 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} serotonin receptors and structure-activity relationships of a series of cyproheptadine analogues. *Chemical and Pharmaceutical Bulletin*, **45**(5), 842–848.
- Hounsgaard, J., Hultborn, H., Jespersen, B., & Kiehn, O. (1988). Bistability of alpha-motoneurons in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *The Journal of Physiology*, **405**(1), 345–367.
- Hounsgaard, J., & Kiehn, O. (1985). Ca⁺⁺ dependent bistability induced by serotonin in spinal motoneurons. *Experimental Brain Research*, **57**, 422–425.
- Hounsgaard, J., & Kiehn, O. (1989). Serotonin-induced bistability of turtle motoneurons caused by a nifedipine-sensitive calcium plateau potential. *The Journal of Physiology*, **414**(1), 265–282.
- Hsiao, C., Trueblood, P., Levine, M., & Chandler, S. (1997). Multiple effects of serotonin on membrane properties of trigeminal motoneurons in vitro. *Journal of Neurophysiology*, **77**(6), 2910–2924.
- Hultborn, H., Denton, M. E., Wienecke, J., & Nielsen, J. (2003). Variable amplification of synaptic input to cat spinal motoneurons by dendritic persistent inward current. *The Journal of Physiology*, **552**(3), 945–952.
- Jacobs, B. L., & Fornal, C. A. (1997). Serotonin and motor activity. *Current Opinion in Neurobiology*, **7**(6), 820–825.
- Jacobs, B. L., Martiñ-Cora, F. J., & Fornal, C. A. (2002). Activity of medullary serotonergic neurons in freely moving animals. *Brain Research Reviews*, **40**(1–3), 45–52.
- Kiehn, O., & Eken, T. (1997). Prolonged firing in motor units: Evidence of plateau potentials in human motoneurons? *Journal of Neurophysiology*, **78**(6), 3061–3068.
- Kuo, J., Lee, R., Zhang, L., & Heckman, C. (2006). Essential role of the persistent sodium current in spike initiation during slowly rising inputs in mouse spinal neurons. *The Journal of Physiology*, **574**(3), 819–834.
- Kuo, J. J., Lee, R. H., Johnson, M. D., Heckman, H. M., & Heckman, C. (2003). Active dendritic integration of inhibitory synaptic inputs in vivo. *Journal of Neurophysiology*, **90**(6), 3617–3624.
- Lee, R., & Heckman, C. (1996). Influence of voltage-sensitive dendritic conductances on bistable firing and effective synaptic current in cat spinal motoneurons in vivo. *Journal of Neurophysiology*, **76**(3), 2107–2110.
- Lee, R., & Heckman, C. (1998a). Bistability in spinal motoneurons in vivo: Systematic variations in persistent inward currents. *Journal of Neurophysiology*, **80**(2), 583–593.
- Lee, R., & Heckman, C. (1998b). Bistability in spinal motoneurons in vivo: Systematic variations in rhythmic firing patterns. *Journal of Neurophysiology*, **80**(2), 572–582.
- Lenth, R., & Lenth, M. R. (2018). Package 'lsmmeans'. *The American Statistician*, **34**(4), 216–221.
- Li, Y., & Bennett, D. J. (2003). Persistent sodium and calcium currents cause plateau potentials in motoneurons of chronic spinal rats. *Journal of Neurophysiology*, **90**(2), 857–869.
- Lipscombe, D., Helton, T. D., & Xu, W. (2004). L-type calcium channels: The low down. *Journal of Neurophysiology*, **92**(5), 2633–2641.
- Ma, J. Y., Catterall, W. A., & Scheuer, T. (1997). Persistent sodium currents through brain sodium channels induced by G protein $\beta\gamma$ subunits. *Neuron*, **19**(2), 443–452.
- Mantegazza, M., Frank, H. Y., Powell, A. J., Clare, J. J., Catterall, W. A., & Scheuer, T. (2005). Molecular determinants for modulation of persistent sodium current by G-protein $\beta\gamma$ subunits. *Journal of Neuroscience*, **25**(13), 3341–3349.
- Mesquita, R. N., Taylor, J. L., Trajano, G. S., Škarabot, J., Holobar, A., Gonçalves, B. A., & Blazevich, A. J. (2022). Effects of reciprocal inhibition and whole-body relaxation on persistent inward currents estimated by two different methods. *The Journal of Physiology*, **600**(11), 2765–2787.
- Murray, K. C., Nakae, A., Stephens, M. J., Rank, M., D'amico, J., Harvey, P. J., Li, X., Harris, R. L., Ballou, E. W., Anelli, R., Heckman, C. J., Mashimo, T., Vavrek, R., Sanelli, L., Gorassini, M. A., Bennett, D. J., & Fouad, K. (2010). Recovery of motoneuron and locomotor function after spinal cord injury depends on constitutive activity in 5-HT_{2C} receptors. *Nature Medicine*, **16**(6), 694–700.
- Murray, K. C., Stephens, M. J., Ballou, E. W., Heckman, C. J., & Bennett, D. J. (2011). Motoneuron excitability and muscle spasms are regulated by 5-HT_{2B} and 5-HT_{2C} receptor activity. *Journal of Neurophysiology*, **105**(2), 731–748.
- Negro, F., Muceli, S., Castronovo, A. M., Holobar, A., & Farina, D. (2016). Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation. *Journal of Neural Engineering*, **13**(2), 026027.
- Orssatto, L. B., Borg, D. N., Blazevich, A. J., Sakugawa, R. L., Shield, A. J., & Trajano, G. S. (2021). Intrinsic motoneuron excitability is reduced in soleus and tibialis anterior of older adults. *GeroScience*, **43**(6), 2719–2735.
- Orssatto, L. B., Fernandes, G. L., & Trajano, G. S. (2022). Facilitation-inhibition control of motor neuronal persistent inward currents in young and older adults. *The Journal of Physiology*, **600**(23), 5101–5117.

- Perrier, J. F., Alaburda, A., & Hounsgaard, J. (2003). 5-HT_{1A} receptors increase excitability of spinal motoneurons by inhibiting a TASK-1-like K⁺ current in the adult turtle. *The Journal of Physiology*, **548**(2), 485–492.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Heisterkamp, S., Van Willigen, B., & Maintainer, R. (2017). Package 'nlme'. *Linear and Nonlinear Mixed Effects Models, Version*, **3**(1), 274.
- Powers, R. K., & Binder, M. D. (2000). Summation of effective synaptic currents and firing rate modulation in cat spinal motoneurons. *Journal of Neurophysiology*, **83**(1), 483–500.
- Schwindt, P., & Crill, W. E. (1980). Properties of a persistent inward current in normal and TEA-injected motoneurons. *Journal of Neurophysiology*, **43**(6), 1700–1724.
- Takahashi, T., & Berger, A. J. (1990). Direct excitation of rat spinal motoneurons by serotonin. *The Journal of Physiology*, **423**(1), 63–76.
- Tenan, M. S., Marti, C. N., & Griffin, L. (2014). Motor unit discharge rate is correlated within individuals: A case for multilevel model statistical analysis. *Journal of Electromyography and Kinesiology*, **24**(6), 917–922.
- Thorstensen, J. R., Taylor, J. L., & Kavanagh, J. J. (2021). Human corticospinal-motoneuronal output is reduced with 5-HT₂ receptor antagonism. *Journal of Neurophysiology*, **125**(4), 1279–1288.
- Thorstensen, J. R., Taylor, J. L., & Kavanagh, J. J. (2022). 5-HT₂ receptor antagonism reduces human motoneuron output to antidromic activation but not to stimulation of corticospinal axons. *European Journal of Neuroscience*, **56**(1), 3674–3686.
- Vandenberk, M. S., & Kalmar, J. M. (2014). An evaluation of paired motor unit estimates of persistent inward current in human motoneurons. *Journal of Neurophysiology*, **111**(9), 1877–1884.
- Veasey, S. C., Fornal, C., Metzler, C., & Jacobs, B. L. (1995). Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. *Journal of Neuroscience*, **15**(7), 5346–5359.
- Wei, K., Glaser, J. I., Deng, L., Thompson, C. K., Stevenson, I. H., Wang, Q., Hornby, T. G., Heckman, C. J., & Kording, K. P. (2014). Serotonin affects movement gain control in the spinal cord. *Journal of Neuroscience*, **34**(38), 12690–12700.
- Wu, G. Y., Han, X. H., Zhuang, Q. X., Zhang, J., Yung, W. H., Chan, Y. S., Zhu, J. N., & Wang, J. J. (2012). Excitatory effect of histamine on rat spinal motoneurons by activation of both H₁ and H₂ receptors in vitro. *Journal of Neuroscience Research*, **90**(1), 132–142.
- Yu, Z., Guindani, M., Grieco, S. F., Chen, L., Holmes, T. C., & Xu, X. (2021). Beyond t test and ANOVA: Applications of mixed-effects models for more rigorous statistical analysis in neuroscience research. *Neuron*, **110**(1), 21–35.

Additional information

Data availability statement

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

Competing interests

All authors declare no conflicts of interest.

Author contributions

B.I.G., S.A.H., A.D.V. and J.J.K. conceptualised and designed the research; B.I.G. performed the experiments; B.I.G., S.A.H., A.D.V. and J.J.K. analysed the data; B.I.G., S.A.H., A.D.V. and J.J.K. interpreted the results of experiments; B.I.G. prepared the figures; B.I.G., S.A.H., A.D.V. and J.J.K. drafted the manuscript; B.I.G., S.A.H., A.D.V. and J.J.K. revised the final version of the manuscript. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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