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RESEARCH REPORT

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5-HT_2 receptor antagonism reduces human motoneuron output to antidromic activation but not to stimulation of corticospinal axons

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Abstract

The intrinsic electrical properties of motoneurons strongly affect motoneuron excitability to fast-acting excitatory ionotropic inputs. Serotonin (5-HT) is a neurochemical that alters the intrinsic properties of motoneurons, whereby animal models and in vitro experiments indicate that 5-HT increases motoneuron excitability by activating 5-HT_2 receptors on the somato-dendritic compartment. In the current study, we examined how antagonism of the 5-HT_2 receptor affects motoneuron excitability in humans. We hypothesised that motoneuron excitability would be reduced. The 5-HT_2 antagonist cyproheptadine was administered to 10 healthy participants in a double-blinded, placebo-controlled, crossover trial. Electrical cervicomedullary stimulation was used to deliver a synchronised excitatory volley to motoneurons to elicit cervicomedullary motor evoked potentials (CMEPs) in the surface electromyography (EMG) signal of the resting biceps brachii. Likewise, electrical peripheral nerve stimulation was used to generate antidromic spikes in motoneurons and cause recurrent discharges, which were recorded with surface EMG as F-waves in a resting hand muscle. Compared with placebo, we found that 5-HT_2 antagonism reduced the amplitude and persistence of F-waves but did not affect CMEP amplitude. 5-HT_2 antagonism also reduced maximal contraction strength. The reduced recurrent discharge of motoneurons with 5-HT_2 antagonism suggests that 5-HT_2 receptors modulate the electrical properties of the initial segment or soma to promote excitability. Conversely, as cyproheptadine did not affect motoneuron excitability to brief synaptic input, but affected maximal contractions requiring sustained input, it seems likely that the 5-HT_2-mediated amplification

Abbreviations: 5-HT, serotonin; ADM, abductor digiti minimi; CMEP, cervicomedullary motor evoked potential; CNS, central nervous system; EMG, electromyography; EPSP, excitatory post-synaptic potential; M_{max}, maximal compound muscle action potential; MVC, maximal voluntary contraction; PICs, persistent inward currents; SSRI, selective serotonin reuptake inhibitor.

of synaptic input at motoneuron dendrites is functionally significant only when excitatory input activates persistent inward currents.

KEYWORDS
cervicomedullary motor evoked potential, F-wave, ionotropic, neuromodulation, raphe nuclei, serotonin

1 | INTRODUCTION

The intrinsic electrical properties of motoneurons markedly influence the magnitude of excitatory post-synaptic potentials (EPSPs) arising from fast-acting excitatory ionotropic inputs (Heckman & Enoka, 2012). Motoneuronal properties are heavily affected by the release of neuromodulators arising from descending brainstem projections (Heckman et al., 2009; Heckman & Enoka, 2012; Rekling et al., 2000). Serotonin (5-HT) is an example of a neuromodulator that has strong effects on motoneuron excitability (i.e., the output of motoneurons to excitatory input). The release of central nervous system (CNS) 5-HT is predominantly controlled by the brainstem raphe nuclei (Hornung, 2003), but there are also intraspinal 5-HT producing neurons (Zhang, 2015). Descending raphe projections form direct monosynaptic connections with the dendrites and soma of motoneurons (Alvarez et al., 1998; Pilowsky et al., 1990; Ridet et al., 1994). At motoneurons, 5-HT enhances the efficacy of ionotropic inputs through the activation of metabotropic receptors coupled to G-proteins (Heckman et al., 2009; Perrier et al., 2013). Specifically, metabotropic 5-HT receptors modulate the electrical properties of motoneurons to promote recruitment and discharge, so that motoneurons are brought above their threshold for discharge, and discharge more frequently, with smaller magnitudes of ionotropic input (Perrier et al., 2013).

Experiments performed in adult turtle spinal cord slice preparations demonstrate that 5-HT increases motoneuron excitability by activating 5-HT_2 receptors on the somatodendritic compartment (Hsiao et al., 1997; Perrier & Hounsgaard, 2003). The facilitatory effects of 5-HT_2 receptor activation on dendritic persistent inward currents (PICs) are especially strong (Harvey et al., 2006a, 2006b; Murray, Stephens, Ballou, et al., 2011; Perrier & Delgado-Lezama, 2005; Perrier & Hounsgaard, 2003). PICs amplify ionotropic input significantly and promote the sustained discharge of motoneurons, and the amplitude of PICs appear to correspond closely to the amount of 5-HT available to activate 5-HT_2 receptors on motoneurons (Heckman et al., 2003, 2009; Heckman, Hyngstrom, & Johnson, 2008). It is important to highlight that a sustained depolarizing current is usually needed to activate PICs (Heckman, Johnson, et al., 2008). In voluntary contractions, this comprises sustained ionotropic input from the corticospinal system, and other descending and sensory afferent pathways, that slowly brings motoneurons above their threshold for discharge. In this regard, 5-HT puts PICs in a facilitated state but does not cause motoneuronal activation, and 5-HT-mediated increases in motoneuron excitability are strongest when PICs are activated by excitatory input (Heckman et al., 2009).

In addition to facilitatory effects on PICs, which are activated by sustained excitatory input and enhance motoneuron output, 5-HT_2 receptors also modulate the resting membrane potential of motoneurons to increase excitability (Perrier et al., 2013; Perrier & Cotel, 2015). Specifically, 5-HT_2 receptors inhibit leak potassium channels and facilitate a hyperpolarization activated inward current (Hsiao et al., 1997). Together, these actions depolarize the resting membrane potential of motoneurons to lower the magnitude of ionotropic input needed for recruitment. If 5-HT increases the excitability of motoneurons in slice preparations and animal models, 5-HT could also increase the in vivo excitability of motoneurons in human participants. In support of this proposition, studies using drug interventions indicate that 5-HT_2 receptors are particularly important and indeed act to increase human motoneuron excitability. For example, 5-HT_2 antagonists reduce spasticity after spinal cord injury by blocking constitutive 5-HT_2 receptor activity at motoneurons (D'Amico, Murray, et al., 2013; Murray et al., 2010). Likewise, in neurologically healthy individuals, 5-HT_2 antagonism reduces the amplitude of muscle responses to motor cortical stimulation (Thorstensen et al., 2021), decreases estimates of PIC amplitude (D'Amico, Murray, et al., 2013) and reduces force variability (Wei et al., 2014). However, motor cortical stimulation and voluntary contractions first activate sites upstream of motoneurons (that are also affected by 5-HT active drugs), so it is not known if/how 5-HT_2 antagonism affects human motoneuron excitability directly.

The purpose of this study was to examine if antagonism of the 5-HT_2 receptor affects human motoneuron excitability. Under conditions of 5-HT_2 antagonism, submaximal stimulation of corticospinal axons at the level of

the cervicomedullary junction was used to deliver a synchronised excitatory volley to motoneurons and evoke cervicomedullary motor evoked potentials (CMEPs) in the resting biceps brachii. Cervicomedullary stimulation rapidly brings motoneurons above their threshold for discharge and causes motoneurons to discharge only once (Berardelli et al., 1991), such that CMEPs are unlikely to reflect the 5-HT_2 receptor modulation of PICs. We also used supramaximal stimulation of motoneuron axons to generate antidromic spikes and cause the recurrent discharge of motoneurons, which were recorded as F-waves in a resting hand muscle. F-waves do not reflect the synaptic activation of motoneurons and are probably sensitive to the excitability of the axon initial segment and soma (McNeil et al., 2013). Compared with placebo, we hypothesised that both the synaptic and antidromic activation of motoneurons would generate muscle responses that were smaller in amplitude and less persistent for F-waves, after administration of a 5-HT_2 antagonist.

As serotonergic projections to the spinal cord are diffuse (Bowker et al., 1982; Heckman, Hyngstrom, & Johnson, 2008; Holstege & Kuypers, 1987) and voluntary motor activity causes 5-HT release to the spinal cord (Jacobs et al., 2002; Veasey et al., 1995; Wei et al., 2014), we also examined CMEPs after a strong contraction of the contralateral limb and hypothesised that 5-HT_2 receptor antagonism would lead to greater reductions in motoneuron excitability at this time because of the stronger influence of 5-HT. Maximal contraction performance was also assessed to determine if 5-HT_2 receptor antagonism affected voluntary motor output. Overall, we found that F-waves but not CMEPs were reduced by 5-HT_2 antagonism, thus indicating that 5-HT_2 antagonism reduces motoneuronal recurrent discharge but not excitability to brief synaptic input. We also found that 5-HT_2 antagonism reduced maximal contraction strength, which suggests that the 5-HT-mediated amplification of synaptic input at motoneurons is strong only when motoneurons fire repetitively and dendritic PICs are activated.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This study was a human, double-blind, placebo-controlled, counterbalanced crossover study. Participants attended the laboratory on two occasions separated by a minimum of 1 week. At one testing session, cyproheptadine was administered to examine how 5-HT_2 antagonism affects the excitability of motoneurons projecting to resting muscle. Measures of motoneuron excitability were obtained before (baseline) and after (post-pill) placebo and drug administration during the two testing days.

2.2 | Ethical approval

Ethical approval was obtained via the Human Research Ethics committee at Griffith University (Griffith University Reference Number: 2019/339), and written informed consent was obtained prior to testing. All testing procedures were performed in accordance with the *Declaration of Helsinki*.

2.3 | Participants

Twelve healthy participants were initially recruited for this study. Participants were screened using a medical history questionnaire which contained exclusion criteria specific to nervous system and upper-limb musculoskeletal injury, cyproheptadine administration and electrical stimulation. Recruited participants also attended a familiarisation session, where we ensured that participants could tolerate cervicomedullary stimulation and that biceps brachii responses to cervicomedullary stimulation were free of early latency components. An early onset latency of an evoked potential from cervicomedullary stimulation signifies the direct stimulation of cervical nerve roots (see Section 2.6). During familiarisation, three participants displayed early latency components to cervicomedullary stimulation and were excluded from testing because of this. The remaining nine participants completed both the cervicomedullary stimulation and F-wave components of the study, and one excluded participant opted to complete the F-wave component only ($n = 10$ were administered drug, participant age: 26.3 $\pm$ 4.1 years old, three female). Participants were asked to refrain from any stimulants or depressants such as caffeine, alcohol or exercise on the morning of testing until testing had finished for the day.

2.4 | Drug intervention

A single 8-mg oral dose of the 5-HT_2 receptor antagonist cyproheptadine, or a placebo, was administered to participants immediately after baseline measurements were obtained. Post-pill testing commenced after 3 h had passed from pill administration. The drug and placebo capsules were similar in appearance, where the placebo contained non-active excipients frequently used in drug compounding. Although also an antihistamine, cyproheptadine has strong antiserotonin effects. In human

neurophysiology experiments, a standardised 8-mg dose of cyproheptadine has been used to attenuate the post-synaptic effects of the 5-HT_2 receptor at motoneurons (D'Amico, Murray, et al., 2013; Murray et al., 2010; Seo et al., 2011; Thorstensen et al., 2021; Wei et al., 2014).

2.5 | Participant setup and EMG

Participants sat upright in a chair with both arms by their side. Both elbows were positioned in ~90° of flexion, and the right wrist was supinated with the palm upwards. The right arm for each participant was firmly strapped to an armrest at the elbow, wrist and fingers to prevent changes in upper-limb position during testing. Surface electromyography (EMG) signals were obtained from the right biceps brachii and abductor digiti minimi (ADM) via Ag/AgCl electrodes (Kendall ARBO, 24 mm diameter). A belly-tendon arrangement was employed for both the biceps brachii and ADM. All biceps brachii and ADM EMG were amplified (×100 or 300) and bandpass filtered between 10 and 1000 Hz (CED 1902, Cambridge Electronic Design Ltd., UK), and sampled at 5000 Hz using a Power 1401 data acquisition interface with Signal software (version 6, Cambridge Electronic Design Ltd., UK). For the ADM, an additional channel was used to collect EMG for F-wave analysis. This channel was highly amplified (×1000) and highly filtered (bandpass between 200 and 1000 Hz) to reduce the duration of the M-wave tail such that it was easier to identify F-waves (D'Amico et al., 2017; Khan et al., 2012, 2016).

2.6 | Cervicomedullary stimulation

Electrical stimuli were applied to corticospinal axons at the level of the cervicomedullary junction to elicit CMEPs in the resting biceps brachii. This muscle was chosen as the threshold for eliciting resting biceps CMEPs is typically lower compared with distal muscles of the upper limb, thus minimising participant discomfort associated with higher intensity cervicomedullary stimulation. A constant current stimulator (DS7AH, Digitimer Ltd., UK) was used to deliver stimuli with a pulse width set at .2 ms. A surface cathode was placed overlying the left mastoid process, and a surface anode was placed overlying the right mastoid process. This configuration generated a current that was less likely to activate axons of cervical motoneurons projecting to the right biceps, as depolarization of peripheral nerves are more probable near the cathode. For the placebo and cyproheptadine sessions, stimulator intensity was set to a level that elicited a resting biceps brachii CMEP amplitude of ~10% of the biceps maximal compound muscle action potential (M_{max}, range: 105–215 mA). To assess the effect of drug administration on CMEPs, this stimulation intensity was fixed for the remainder of each testing session. The onset latency of CMEPs was monitored throughout testing to ensure activation of biceps brachii motoneurons via synaptic input. The onset latency for biceps CMEPs is typically ≥8 ms (Taylor & Martin, 2009; Ugawa et al., 1991). Likewise, a sudden ~2-ms decrease in latency suggested that cervicomedullary stimulation activated the axons of cervical motoneurons (Petersen et al., 2002; Taylor, 2006; Taylor & Gandevia, 2004).

2.7 | Brachial plexus stimulation

Electrical stimuli were applied to axons of biceps brachii motoneurons at the brachial plexus to generate a biceps M_{max}. A constant current stimulator (DS7AH, Digitimer Ltd., UK) with a pulse width of .2 ms was used to deliver stimulation. A surface cathode was positioned overlying the supraclavicular fossa at Erb's point, and a surface anode was positioned on the acromion. For the placebo and cyproheptadine sessions, stimulator intensity was set at 130% of the current used to obtain a maximal evoked biceps brachii EMG response before pill ingestion (range: 52–156 mA). Once this stimulator intensity was determined, it remained fixed throughout the testing session.

2.8 | Ulnar nerve stimulation

Because the appearance of F-waves in the biceps brachii EMG signal can be masked by the tail of the preceding M-wave, we selected a distal upper-limb muscle to record F-waves with a longer onset latency. Hence, electrical stimuli were applied to the ulnar nerve at the wrist to generate F-waves in the ADM. A constant current stimulator (DS7AH, Digitimer Ltd., UK) with a pulse width of .2 ms was used to generate the antidromic wave in the ulnar nerve. A surface cathode and anode were positioned ~2 cm apart, where the anode was positioned distal to the cathode and ~2–3 cm proximal to the wrist joint. All ulnar nerve stimuli were supramaximal (150% of the current used to obtain a resting ADM M_{max}, range: 45–105 mA). This supramaximal intensity was determined prior to pill ingestion and remained fixed within each testing session. There was a 2-s interval (.5-Hz stimulation frequency) between each ulnar nerve stimulation.

2.9 | Experiment protocol

After the participant was prepared for testing, and relevant stimulation intensities were determined, three blocks of baseline stimulations were completed (Figure 1). Within each block, five cervicomedullary stimuli were used to obtain CMEPs and three brachial plexus stimuli were used to obtain M_{max} for the biceps brachii. There were 10-s inter-stimulus intervals between all cervicomedullary and brachial plexus stimuli. Each block also contained 60 ulnar nerve stimuli. Following baseline measurements, participants ingested a placebo or cyproheptadine. Three hours after pill administration, a further five stimulation blocks were obtained to assess post-pill changes in evoked responses. All stimulation blocks were separated by 5 min. Before baseline and post-pill testing, we ensured participants were in a comfortable position before measurements were obtained, and we asked participants to rest with no movement for ~5 min to ensure they were relaxed before stimulations were delivered. Throughout testing, participants were instructed to minimise voluntary movement, and EMG was monitored during stimulation blocks to ensure muscles were free of voluntary activity.

Additional data were collected to determine if remote contraction of the contralateral limb affected CMEPs under conditions of 5-HT_2 antagonism. Voluntary contraction of a remote muscle group, such as the opposite non-test limb, likely promotes diffuse serotonergic drive to the spinal cord, which will affect nearby motoneuron pools innervating contralateral homologous muscles (e.g., the biceps of the test limb) (Wei et al., 2014). All muscle contractions in this experiment were performed at the end of testing on both days so that voluntary activity did not affect baseline and post-pill measures of resting motoneuron excitability. For all contractions, the wrist of the left limb was attached to a calibrated load cell (~1100-N capacity, SM-250 S-type, Interface Inc., USA) via a non-compliant strap. Participants initially performed three brief (~2 to 3 s) maximal voluntary contractions (MVCs) to obtain an estimate of maximal force generating capability for the non-test limb. For MVCs, real-time visual feedback of force and verbal encouragement were provided to ensure each participant was contracting at maximal effort. After peak MVC force was established, participants generated five brief (~4 s) isometric elbow flexions at 75% MVC, where each contraction was followed by cervicomedullary and brachial plexus stimulations approximately 1 and 3 s after contraction. EMG responses to cervicomedullary and brachial plexus stimulation were measured in the relaxed right biceps brachii directly after each contraction. Participants were instructed to immediately relax both limbs after contraction of the left limb so that all stimulations were applied when the right biceps brachii was at rest. Biceps EMG was monitored to ensure the elbow flexors of the test limb were relaxed. All contractions were separated by 2 min to minimise fatigue and standardise rest periods.

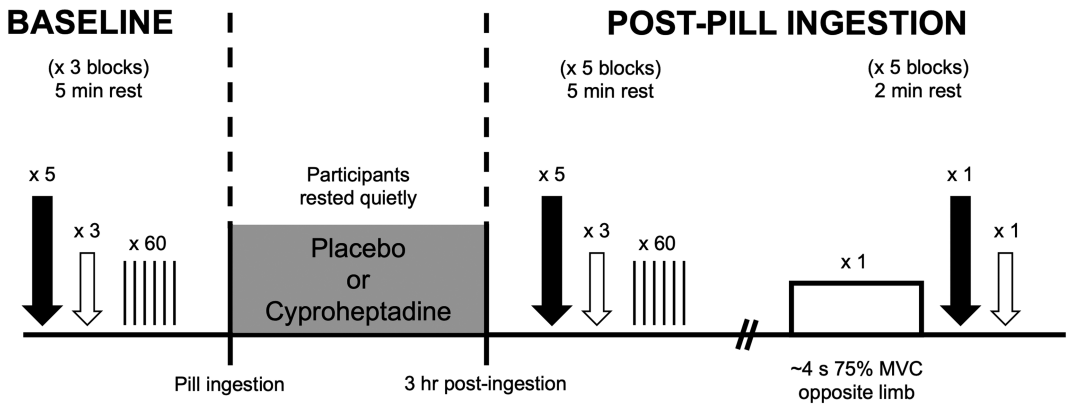

FIGURE 1 Experiment protocol used to assess motoneuron excitability. Participants attended two sessions where a placebo or cyproheptadine was administered. Baseline and post-pill measures were made for cervicomedullary motor evoked potentials (CMEPs, closed arrows) and F-waves (clustered vertical lines). Brachial plexus stimuli were also used to obtain biceps maximal compound muscle action potentials (M_{max}, open arrows). Three blocks of baseline stimulations and five blocks of post-pill stimulations were delivered. Each block included 5 biceps CMEPs, 3 biceps M_{max}, and 60 stimuli for F-waves. Blocks were separated by 5 min. After resting measurement blocks were completed, brief submaximal elbow flexions (at 75% of maximal voluntary contraction, MVC) were performed with the contralateral limb. Directly after each contraction of the contralateral limb, responses to cervicomedullary and brachial plexus stimulation were recorded (single submaximal contraction and stimulations = 1 block). Five submaximal contraction blocks were completed for post-ingestion conditions separated by 2 min

2.10 | Data analysis

The peak-to-peak amplitudes of CMEPs and M_{max} were calculated from the biceps EMG signal. Average CMEP amplitudes were first normalised to average M_{max} amplitudes, and post-pill CMEP amplitudes were normalised to baseline amplitudes to account for between-session differences. The peak-to-peak amplitudes of F-waves were extracted from heavily filtered EMG data and normalised to heavily filtered M_{max} amplitudes. Average post-pill F-wave amplitudes were then normalised to baseline amplitudes. In addition to amplitude measures, F-wave persistence was calculated by counting the number of times an F-wave was present relative to the number of ulnar nerve stimuli. We deemed an F-wave to be present if there was a clear waveform response with an amplitude ≥ 20 μV within the 25- to 50-ms time window after stimulation. Baseline F-wave persistence values were subtracted from post-pill persistence values so that a negative value represents a reduction from baseline. All CMEP and F-wave data are presented in figures as either a percentage (%) of baseline or change from baseline. Change scores were also calculated for post-pill measures as a difference from placebo values (cyproheptadine–placebo), and these change scores are also presented in figures. A negative change score denotes a reduction in amplitude or persistence for the cyproheptadine condition relative to placebo. All data were analysed using Signal software (version 6, Cambridge Electronic Design Ltd., UK).

2.11 | Statistical analysis

Shapiro–Wilk tests were used to assess normality. To identify differences between drug conditions, two-tailed paired samples *t* tests were employed for peak MVC force, CMEP amplitude and F-wave amplitude. Data were not normally distributed for F-wave persistence,

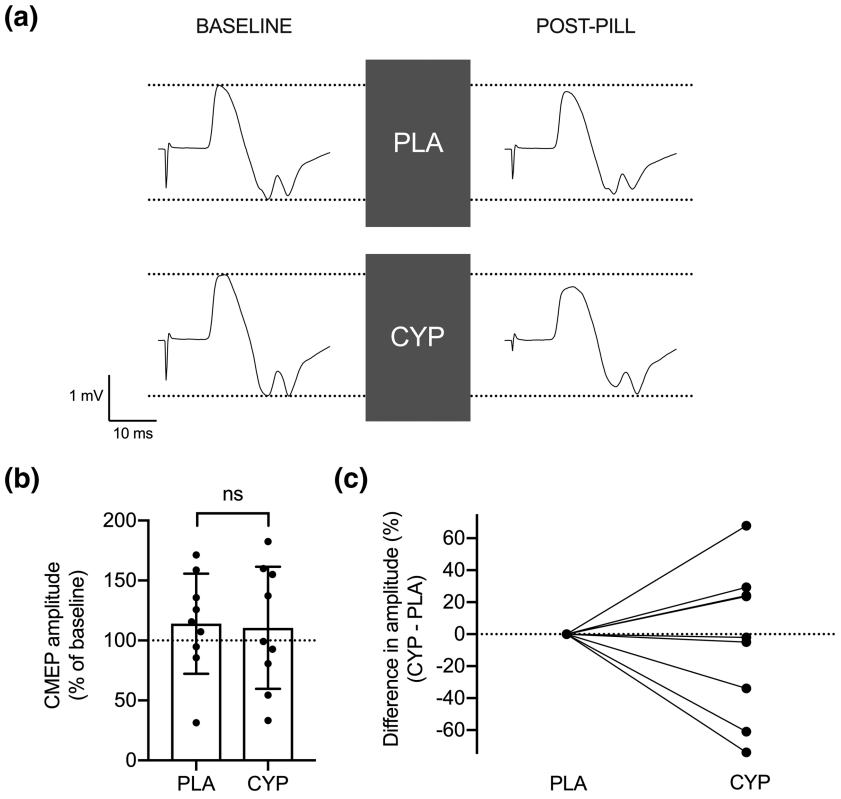

FIGURE 2 Changes in biceps brachii cervicomedullary motor evoked potential (CMEP) amplitude after placebo (PLA) and cyproheptadine (CYP) administration. (a) Waveform averages of biceps CMEPs are shown for a single participant before (baseline) and after (post-pill) administration of placebo (top panel) and cyproheptadine (bottom panel). Baseline traces are the average of 15 CMEPs and post-pill traces are the average of 25 CMEPs. Dotted horizontal traces denote the maximum and minimum waveform responses at baseline for each drug condition. (b) Post-pill CMEP amplitudes are shown for the group (mean ± *SD*, $n = 9$). Individual values for each participant are also shown. CMEP amplitudes were first normalised to the amplitude of the maximal compound muscle action potential (M_{max}), and then normalised to baseline CMEP (% of M_{max}) values. (c) Individual post-pill CMEP amplitudes are shown for the cyproheptadine condition, where cyproheptadine amplitudes are presented as a difference from post-pill placebo values to show the direction of change for each participant after drug intake (cyproheptadine minus placebo)

TABLE 1 Biceps brachii and abductor digiti minimi (ADM) maximal compound muscle action potential (M_{max}) amplitude values

	Resting muscle				Post-contralateral contraction	
	Pre-PLA	**Pre-CYP**	**Post-PLA**	**Post-CYP**	**Post-PLA**	**Post-CYP**
Biceps brachii (mV)	18.3 ± 6.8	18.0 ± 8.4	19.4 ± 7.4	19.6 ± 9.3	19.2 ± 7.7	19.7 ± 9.2
ADM (mV)	17.3 ± 2.4	18.0 ± 2.1	17.9 ± 2.2	17.8 ± 2.3	—	—

Note: ADM is abductor digiti minimi, PLA is placebo, and CYP is cyproheptadine. ADM M-waves were obtained from wide band pass EMG signals. Data are presented as group means ± *SD* ($n = 9$ for biceps brachii and $n = 10$ for ADM).

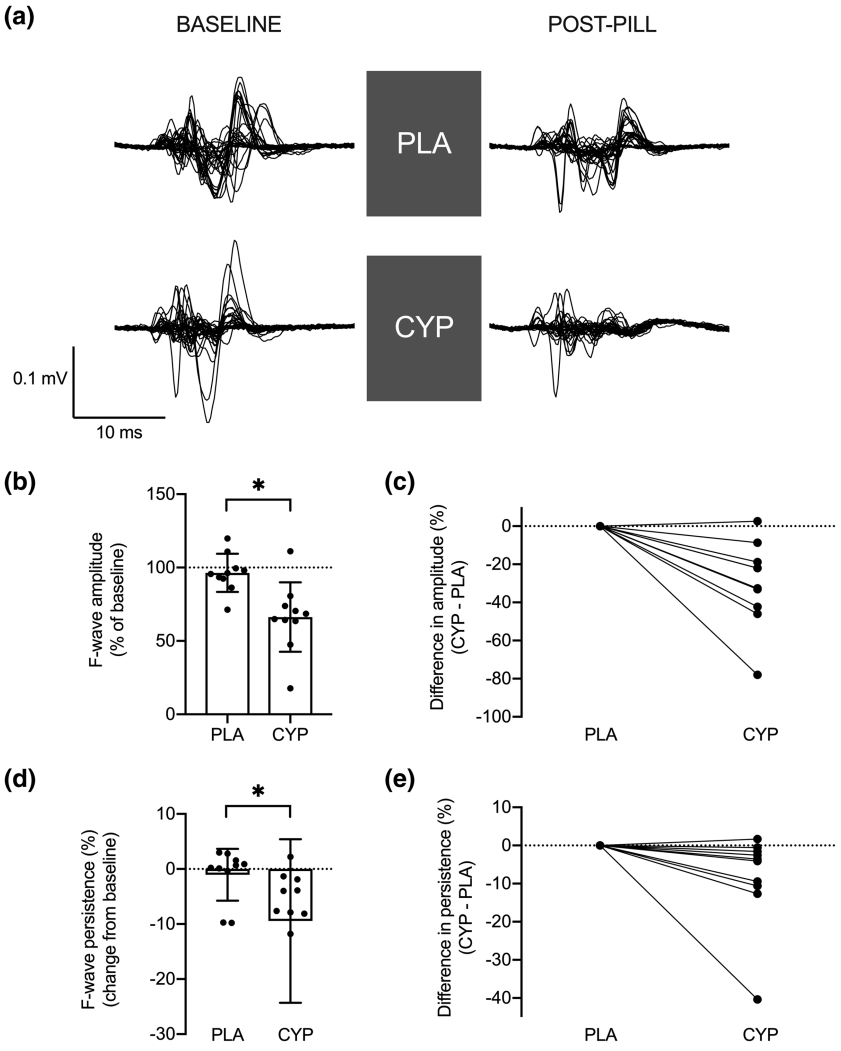

FIGURE 3 Changes in abductor digiti minimi F-wave amplitude and persistence after placebo (PLA) and cyproheptadine (CYP) administration. (a) Individual F-waves are shown for a single participant before (baseline) and after (post-pill) administration of placebo (top panel) and cyproheptadine (bottom panel). Thirty consecutive responses to stimulation are overlaid for each collection of traces. F-waves are shown without the preceding M-wave, and each trace is the time window from 24 to 50 ms after stimulation. (b) Post-pill F-wave amplitudes are shown for the group. Individual values for each participant are also shown. Individual F-wave amplitudes were first normalised to the amplitude of the maximal compound muscle action potential (M_{max}), and then normalised to baseline F-wave (% of M_{max}) values. (c) Individual post-pill F-wave amplitudes are shown for the cyproheptadine condition, where cyproheptadine amplitudes are presented as a difference from post-pill placebo values to show the direction of change for each participant after drug intake (cyproheptadine minus placebo). (d) Change in post-pill F-wave persistence values are shown for the group. Individual values for each participant are also shown. For one participant, their cyproheptadine value (−50.1%) is outside the y-axis limits. (e) Individual post-pill F-wave persistence values are shown for the cyproheptadine condition, where cyproheptadine persistence values are presented as a difference from post-pill placebo values. For panels (b) and (d), values are mean ± *SD* ($n = 10$), and '*' denotes a significant difference between drug conditions ($P < .05$)

and a Wilcoxon signed-rank test was used. All statistical tests were completed using SPSS Statistics (version 27, IBM Corp., USA). A P value of <.05 was considered statistically significant. All group data are presented in the text and figures as mean ± *SD*.

3 | RESULTS

3.1 | Cervicomedullary motor evoked potentials

Waveform averages of raw biceps CMEPs are shown for a single participant in Figure 2a. Before pill administration, the amplitude of baseline CMEPs for the group was 9.7 ± 5.7% of M_{max} for the placebo session and 8.1 ± 3.4% of M_{max} for the cyproheptadine session. Biceps M_{max} amplitudes are presented in Table 1. Post-pill CMEP amplitude was not different between the placebo and cyproheptadine conditions ($t_8 = .22$, $P = .83$, Figure 2b). Figure 2c shows individual change from placebo values for post-pill cyproheptadine CMEP amplitude data.

3.2 | F-waves

Raw ADM F-waves are shown for a single participant in Figure 3a. Before pill administration, baseline F-wave amplitude for the group was 2.6 ± .6% of M_{max} for the placebo session and 2.5 ± .8% of M_{max} for the cyproheptadine session. ADM M_{max} amplitudes are presented in Table 1. Baseline F-wave persistence was high. For the group, persistence was 95.9 ± 2.8% for the placebo session and 91.6 ± 9.4% for the cyproheptadine session. Cyproheptadine administration had a strong effect on both F-wave amplitude and persistence, whereby drug administration reduced both measures (cyproheptadine

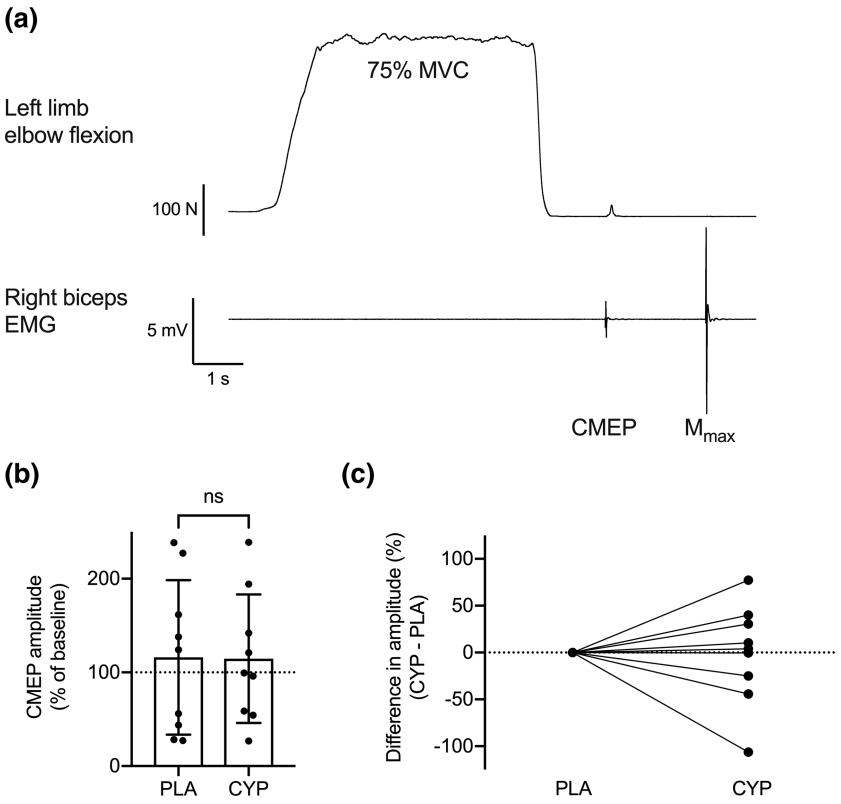

FIGURE 4 Biceps brachii cervicomedullary motor evoked potential (CMEP) amplitude after a brief contraction of the contralateral elbow flexors with placebo (PLA) and cyproheptadine (CYP) administration. (a) Raw traces of elbow flexion force generated from the left limb (upper trace) and biceps EMG recorded from the right limb (lower trace). Participants generated brief elbow flexions of the left limb at 75% of maximal voluntary contraction (MVC), before receiving cervicomedullary and brachial plexus stimulations at rest to evoke muscle responses in the right biceps. (b) Post-contraction CMEP amplitudes are shown for the group (mean ± *SD*, $n = 9$). Individual values for each participant are also shown. CMEP amplitudes were first normalised to the amplitude of the maximal compound muscle action potential (M_{max}, also post-contraction), and then normalised to baseline pre-pill CMEP (% of M_{max}) values. (c) Individual post-contraction CMEP amplitudes are shown for the cyproheptadine condition, where cyproheptadine amplitudes are presented as a difference from post-contraction placebo values to show the direction of change for each participant after drug intake (cyproheptadine minus placebo)

amplitude: $-30.1 \pm 22.3\%$ difference from placebo, cyproheptadine persistence: $-8.4 \pm 12.2\%$ difference from placebo). The amplitude of post-pill F-waves was significantly different between the placebo and cyproheptadine conditions ($t_9 = 4.26$, $P = .002$, Figure 3b). Similarly, post-pill F-wave persistence was different between drug conditions ($Z = -2.50$, $P = .013$, Figure 3d). Figure 3c,e shows individual change from placebo values for post-pill cyproheptadine amplitude and persistence data, respectively.

3.3 | The effects of contralateral contraction on CMEPs

After resting measures had been obtained, participants performed brief MVCs of the left contralateral elbow flexors to determine relevant target contraction intensities for the additional experiment and to establish if the cyproheptadine intervention affected maximal motor output. Indeed, we identified a difference between drug conditions for MVC force ($t_9 = 2.97$, $P = .016$), where MVC force for the cyproheptadine condition (284.6 ± 74.5 N) was less than placebo (303.6 ± 88.5 N). Although maximal motor output was affected by drug administration, cyproheptadine did not affect CMEP amplitude measured in the right limb when cervicomedullary stimuli were delivered immediately after submaximal contraction of the left limb (Figure 4). After submaximal elbow flexion of the left contralateral limb, there was no difference between the placebo and cyproheptadine conditions for biceps CMEP amplitude ($t_8 = .084$, $P = .93$).

4 | DISCUSSION

This study examined if 5-HT_2 receptor antagonism affects human motoneuron excitability. Specifically, we assessed the effects of 5-HT_2 antagonism on the synaptic activation of motoneurons via cervicomedullary stimulation, and the antidromic activation and recurrent discharge of motoneurons via peripheral nerve stimulation. Cervicomedullary stimulation delivers a synchronised excitatory volley to motoneurons to evoke a compound muscle action potential but does not cause motoneurons to discharge repetitively and is unlikely to activate PICs. We found that muscle responses from cervicomedullary stimulation were not affected by 5-HT_2 antagonism, but 5-HT_2 antagonism reduced recurrent motoneuron discharge. We also assessed the synaptic activation of motoneurons after a strong contraction of the contralateral limb and found that 5-HT_2 antagonism did not affect muscle responses to cervicomedullary stimulation after remote muscle activity. Yet 5-HT_2 antagonism reduced maximal contraction strength (i.e., when synaptic input generates repetitive firing of motoneurons to activate PICs). Overall, these results provide novel, in vivo, human evidence that 5-HT_2 receptors control the excitability of motoneurons, as evidenced by changes in recurrent discharge after drug administration. In addition, our results indicate that 5-HT_2 receptors modulate responses to synaptic input, but only when synaptic input activates 5-HT_2 receptor mediated dendritic PICs.

4.1 | 5-HT_2 antagonism does not affect CMEPs

We used cervicomedullary stimulation to deliver a brief excitatory volley to motoneurons and recorded the size of the resultant EMG response. Stimulation at the cervicomedullary junction provided a way to assess motoneuron output in response to synaptic input from descending motor pathways (McNeil et al., 2013; Taylor, 2006; Taylor & Gandevia, 2004), where CMEPs have a strong monosynaptic component for the biceps brachii (Petersen et al., 2002). As stimulation intensity was fixed from baseline, cervicomedullary stimulation activated the same number/type of corticospinal axons and caused similar excitation of motoneurons throughout each session. However, even though the synaptic excitation of motoneurons was controlled, 5-HT_2 antagonism had no effect on the output of motoneurons to input provided by a single synchronous volley from descending motor pathways.

Although 5-HT_2 receptors modulate the resting membrane properties of motoneurons to enhance motoneuron excitability (Hsiao et al., 1997), 5-HT_2 receptors exert their most powerful effects on motoneuron excitability by facilitating voltage-gated PICs (Harvey et al., 2006a, 2006b; Murray, Stephens, Ballou, et al., 2011; Perrier & Delgado-Lezama, 2005; Perrier & Hounsgaard, 2003). However, activation of PICs in motoneurons, and especially activation of calcium PICs (Elbasiouny et al., 2006), requires a sustained synaptic excitation. Thus, 5-HT_2 receptor effects on PICs will not be reflected in the amplitude of muscle responses to single-pulse cervicomedullary stimulation. With stimulation of corticospinal axons, motoneurons receive a brief, synchronised input and quickly go from below to above their threshold for discharge. This leaves little time for PICs to activate and influence the intrinsic properties of motoneurons and hence, the amplitude of the CMEP. In decerebrate cat preparations, self-sustained motoneuron firing can be elicited with a high-frequency 1.5-s activation of Ia afferents that brings motoneurons above their

threshold for discharge but provides sufficient time for PICs to activate (Lee & Heckman, 1998). Likewise, in humans, a high-frequency vibration applied for 8 s to the wrist generates a reflexive response that is enhanced by a selective 5-HT reuptake inhibitor (SSRI), an effect that is likely due to a 5-HT facilitation of PICs (Wei et al., 2014). These methods contrast with cervicomedullary stimulation which generates a single EPSP in motoneurons and does not cause repetitive discharge.

The proposition that a *sustained* depolarizing input is needed to activate PICs, and a reduced PIC facilitation with 5-HT_2 antagonism will not reduce motoneuron excitability to a *brief* synaptic volley, also explains why we identified no drug differences for CMEPs after strong contralateral contraction. In the current study, voluntary contractions of the opposite limb were intended to enhance 5-HT concentration in the spinal cord (Jacobs et al., 2002; Veasey et al., 1995), but PICs in motoneurons projecting to the biceps of the test limb were not active as this muscle was relaxed. We postulate that serotonergic effects after contralateral contraction may be revealed with activation of PICs, for example, if participants voluntarily activate motoneurons projecting to the test biceps. The serotonergic modulation of PICs after remote muscle activity has been indirectly demonstrated in humans. In healthy participants, the variability of muscle contraction force is increased with enhanced 5-HT availability and reduced with 5-HT_2 antagonism, and the magnitude of these increases or decreases in force variability is in proportion to the strength of a preceding voluntary contraction from the contralateral limb (Wei et al., 2014). In this example, changes in force variability were likely mediated by the modulation of PICs by the 5-HT drugs, but PICs were only activated by voluntary contraction. It seems unlikely that the lack of drug effects on post-contraction CMEPs in our study were due to the temporal characteristics of 5-HT reuptake in synapses, as increased motoneuron excitability from 5-HT release has been detected up until 8.5 s after stimulation of the raphe nuclei in turtle preparations (Perrier & Delgado-Lezama, 2005). In our protocol, CMEPs were obtained ~1 s after contraction, which means that the effects of 5-HT release were still present when measurements of motoneuron excitability were made.

4.2 | F-waves are reduced with 5-HT_2 antagonism

Another way to assess motoneuron excitability is to measure the amplitude and persistence of recurrent motoneuron discharge, or F-waves when measured with surface EMG, after supramaximal stimulation of a motor nerve (Fisher, 1992; McNeil et al., 2013; Mesrati & Vecchierini, 2004). F-waves reflect the antidromic activation and subsequent backfiring of motoneurons. Hence, the generation of F-waves does not require synaptic input. We found that 5-HT_2 antagonism reduced the amplitude and persistence of recurrent motoneuron discharge, which demonstrates reductions in motoneuron excitability with reduced 5-HT_2 receptor activity.

Considering that recurrent discharge was generated in motoneurons that are otherwise below their threshold for discharge (prior to stimulation), drug reductions in motoneuron excitability can be attributed to effects on resting membrane properties and not PICs. In guinea pig motoneurons, 5-HT_2 receptor activation increases excitability below recruitment threshold by inhibiting leak potassium channels and facilitating a hyperpolarization activated inward current (Hsiao et al., 1997). In our study, 5-HT_2 antagonism could have combatted these facilitatory effects to reduce motoneuron excitability and reduce F-waves. However, this does not explain why muscle responses to antidromic but not synaptic activation of motoneurons were affected by drug, as overall changes in intrinsic properties of motoneurons affect both measures. One possibility is that drug administration affected the motoneuron soma to reduce the time between antidromic and orthodromic spikes at the axon initial segment. Although speculative, 5-HT_2 antagonism with cyproheptadine could have reduced the amplitude of the persistent sodium current, which is faster to activate than persistent calcium currents (Heckman, Johnson, et al., 2008), to reduce the duration of somatic depolarization. In turn, a shorter duration somatic depolarization would increase the likelihood of an orthodromic spike reaching a refractory axon initial segment. Although there is limited evidence for this idea, 5-HT_2 receptors facilitate sodium PICs in rat motoneurons (Harvey et al., 2006a, 2006b).

Another possibility is that cyproheptadine differently affected different motoneuron types. F-waves represent the recurrent discharge of higher threshold motoneurons, as the simultaneous stimulation of Ia afferents initiates orthodromic spikes in lower threshold motoneurons that collide with the antidromic spikes that generate recurrent discharge (Espiritu et al., 2003). By contrast, stimulation of corticospinal axons recruits motoneurons in an orderly fashion from lower to higher threshold (Bawa & Lemon, 1993). In this regard, as we set the intensity of cervicomedullary stimulation to elicit small CMEPs (~10% of M_{max}), CMEPs in this study reflect the excitability of lower threshold motoneurons. As F-waves but not CMEPs were reduced with drug, this indicates that 5-HT_2 antagonism may have affected higher threshold motoneurons more than lower threshold.

4.3 | 5-HT$_2$ antagonism reduces motor output

Although there is a low level of tonic 5-HT release to motoneurons in the waking state, stimulation assessed motoneuron excitability under conditions with no ionotropic drive to activate motoneurons. However, voluntary motor activity causes greater 5-HT release to motoneurons (Jacobs et al., 2002; Veasey et al., 1995), and neuromodulatory effects at motoneurons are strongest with high-intensity motor activities involving strong serotonergic drive and sustained ionotropic input to activate PICs (Heckman et al., 2003, 2009). Our maximal contraction findings are consistent with this, as 5-HT$_2$ antagonism reduced the force generated during maximal contractions. This is not an accidental finding, as enhanced 5-HT availability increases maximal strength and/or voluntary activation (Kavanagh et al., 2019; Thorstensen et al., 2020), and maximal contractions were also negatively affected with 5-HT$_2$ antagonism in a previous study (Thorstensen et al., 2021). Considering that maximal contractions are generated via sustained input to motoneurons, we suggest that 5-HT$_2$ antagonism reduced the amplitude of PICs to negatively affect the ability of participants to voluntarily activate motoneurons leading to reductions in muscle force.

4.4 | Considerations

Both stimulation techniques assessed a small proportion of the motoneuron pool and thus might not reflect overall changes in excitability, and cyproheptadine may have affected biceps and ADM pools differently. Also, increases or decreases in F-wave amplitude or persistence do not always represent corresponding increases or decreases in motoneuron excitability (Balbi, 2016; Balbi et al., 2014; Lin & Floeter, 2004). As afferent fibres are also activated with supramaximal stimulation of peripheral nerves and 5-HT modulates afferent input to motoneurons (D'Amico, Li, et al., 2013; Murray, Stephens, Rank, et al., 2011), 5-HT effects on F-waves could potentially be a consequence of pre-synaptic mechanisms. However, the serotonergic control of afferent input to human motoneurons is via 5-HT$_{1B/D}$ receptors (D'Amico, Li, et al., 2013), and cyproheptadine has weak effects at these receptors.

5 | CONCLUSION

This study provides novel in vivo evidence that 5-HT$_2$ receptors play a role in controlling the excitability of human motoneurons, as evidenced by reductions in F-waves with 5-HT$_2$ antagonism. Considering that assessments of motoneuron excitability were obtained when muscles were inactive and thus, motoneurons were otherwise below their threshold for discharge, the reduced recurrent discharge of motoneurons with 5-HT$_2$ antagonism suggests that 5-HT modulates the resting membrane properties of the axon initial segment or soma to promote motoneuronal excitability. In addition, as 5-HT$_2$ antagonism had no effect on motoneuron excitability to brief synaptic input, but affected maximal contractions requiring sustained synaptic input, it is likely that the 5-HT-mediated amplification of synaptic input at motoneurons is strong only when dendritic PICs are activated.

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CONFLICT OF INTEREST

None to declare.

AUTHOR CONTRIBUTIONS

JRT, JLT and JJK contributed to the conception and the design of this study. JRT, JLT and JJK contributed to the interpretation, drafting and final approval of the manuscript. Data collection and analysis were performed by JRT at Griffith University.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

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

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