




# Human motoneurone excitability is depressed by activation of serotonin 1A receptors with buspirone

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## Key points

- In the adult turtle spinal cord, action potential generation in motoneurons is inhibited by spillover of serotonin to extrasynaptic serotonin 1A (5-HT<sub>1A</sub>) receptors at the axon initial segment. We explored whether ingestion of the 5-HT<sub>1A</sub> receptor partial agonist, buspirone, decreases motoneurone excitability in humans.
- Following ingestion of buspirone, two tests of motoneurone excitability showed decreases. F-wave areas and persistence in an intrinsic muscle of the hand were reduced, as was the area of cervicomedullary motor evoked potentials in biceps brachii.
- Our findings suggest that activation of 5-HT<sub>1A</sub> receptors depresses human motoneurone excitability. Such a depression could contribute to decreased motoneurone output during fatiguing exercise if there is high serotonergic drive to the motoneurons.

**Abstract** Intense serotonergic drive in the turtle spinal cord results in serotonin spillover to the axon initial segment of the motoneurons where it activates serotonin 1A (5-HT<sub>1A</sub>) receptors and inhibits generation of action potentials. We examined whether activation of 5-HT<sub>1A</sub> receptors decreases motoneurone excitability in humans by determining the effects of a 5-HT<sub>1A</sub> receptor partial agonist, buspirone, on F waves and cervicomedullary motor evoked potentials (CMEPs). In a placebo-controlled double-blind study, 10 participants were tested on two occasions where either placebo or 20 mg of buspirone was administered orally. The ulnar nerve was stimulated supramaximally to evoke F waves in abductor digiti minimi (ADM). CMEPs and the maximal M wave were elicited in biceps brachii by cervicomedullary stimulation and brachial plexus stimulation, respectively. Following buspirone intake, F-wave area and persistence, as well as CMEP area, were significantly decreased. The mean post-pill difference in normalized F-wave areas and persistence between buspirone and placebo days was  $-27\%$  ( $-42, -12$ ; 95% confidence interval) and  $-9\%$  ( $-16, -2$ ), respectively. The mean post-pill difference in normalized CMEP area between buspirone and placebo days showed greater variation and was  $-31\%$  ( $-60, -2$ ). In conclusion, buspirone reduces motoneurone excitability in humans probably via activation of 5-HT<sub>1A</sub> receptors at the axon initial segment. This has implications for motor output during high drive to the motoneurons when serotonin may spill over to these inhibitory receptors and consequently inhibit motoneurone output. Such a mechanism could potentially contribute to fatigue with exercise.

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**Abbreviations** 5-HT, serotonin; 5-HT<sub>1A</sub>, serotonin 1A receptor subtype; ADM, abductor digiti mini; CI, confidence interval; CMEP, cervicomedullary motor evoked potential; EMG, electromyography; FDI, first dorsal interosseous;  $M_{\max}$ , maximal compound muscle action potential.

## Introduction

Serotonin (5-HT) is a neuromodulator that can alter motoneurone output in humans and animal models. Spinal motoneurons receive direct, dense serotonergic innervation of the soma and dendrites from neurons with cell bodies in the medullary raphe nuclei (Carlsson *et al.* 1963; Carlsson *et al.* 1964; Steinbusch 1981; Kiehn *et al.* 1992; Alvarez *et al.* 1998; Schmidt & Jordan, 2000; Hornung, 2003). Increased firing of serotonergic raphe neurons suggests that the release of 5-HT onto motoneurons increases during periods of motor activity (Fornal *et al.* 1985; Veasey *et al.* 1995; Jacobs & Fornal, 1997; Jacobs *et al.* 2002). In various animal models and in humans, 5-HT has been shown to have excitatory effects on intrinsic motoneurone excitability via the activation of G-q coupled 5-HT<sub>2B/C</sub> receptors that are distributed on the dendrites and the soma of the motoneurons (Perrier & Hounsgaard, 2003; Harvey *et al.* 2006a; Harvey *et al.* 2006b; D'Amico *et al.* 2013b). However, in some animal models, 5-HT also directly inhibits motoneurone excitability via G-i coupled 5-HT<sub>1A</sub> receptors located at the axon initial segment (Innis *et al.* 1988; Jackson & White, 1990; Pennington & Kelly, 1990; Cotel *et al.* 2013). The axon initial segment is the site of action potential generation as a result of the high density of voltage-gated sodium channels that act to lower the threshold for action potential initiation (Coombs *et al.* 1957; Duflocq *et al.* 2008; Duflocq *et al.* 2011). Activation of 5-HT<sub>1A</sub> receptors at the axon initial segment inhibits the sodium current responsible for spike initiation, with a consequent decrease in motoneurone output (Cotel *et al.* 2013).

It is difficult to discern the functional implications of serotonin release on motoneurone excitability given the direct opposing actions of 5-HT at the motoneurons. However, recent work in the adult turtle spinal cord has shed light on the mechanisms that allow dual modulation of motoneurone excitability by 5-HT. In the adult turtle spinal cord, brief (~ 1 s) stimulation of the serotonergic dorsolateral funiculus tract produced excitation of the motoneurons through activation of 5-HT<sub>2B/C</sub> receptors, despite the higher affinity of 5-HT for inhibitory 5-HT<sub>1A</sub> receptors (Cotel *et al.* 2013). Unlike motoneurone excitation via the 5-HT<sub>2B/C</sub> receptors, which are in close proximity to serotonergic synapses, 5-HT<sub>1A</sub> mediated inhibition only occurred during prolonged stimulation of the dorsolateral funiculus (3 min) and appears to depend on the spillover of 5-HT to the axon initial segment, which does not receive serotonergic innervation (Cotel *et al.* 2013; Montague *et al.* 2013;

Maratta *et al.* 2015). Therefore, it appears that serotonergic inhibition of the motoneurons only occurs during high serotonergic drive to the motoneurons, whereas overall excitation occurs at lower levels of drive. This novel finding has identified these 5-HT<sub>1A</sub> receptors, which are present in the human spinal cord (Laporte *et al.* 1996), as a potential contributor to central fatigue (Cotel *et al.* 2013). Central fatigue represents the contribution of the nervous system to muscle fatigue (Gandevia, 2001). That is, the progressive decline in the ability to produce voluntary muscle force as exercise continues. In humans, large reductions in the excitability and firing rates of motoneurons occur during and following fatiguing maximal contractions (Bellemare *et al.* 1983; Bigland-Ritchie *et al.* 1983; Gandevia *et al.* 1999; Butler *et al.* 2003; Khan *et al.* 2012). The proposition that findings in the adult turtle spinal cord translate to effects on human motor performance relies on two assumptions: (i) activation of 5-HT<sub>1A</sub> receptors located on human motoneurons depresses motoneurone output and (ii) there is a high level of serotonergic drive to the motoneurons during fatiguing, maximal contractions. In the present study, we aimed to confirm the first assumption, that is, whether activation of 5-HT<sub>1A</sub> receptors with the selective partial agonist, buspirone (Eison & Temple, 1986; Mahmood & Sahajwalla, 1999; Loane & Politis, 2012), depresses motoneurone excitability in healthy individuals. An abstract of our results has been published previously (D'Amico *et al.* 2015).

## Methods

In a double-blind, placebo-controlled study, participants attended the laboratory on two days to determine the effects of buspirone (a 5-HT<sub>1A</sub> receptor partial agonist) (Eison & Temple, 1986; Mahmood & Sahajwalla, 1999; Loane & Politis, 2012) on motoneurone excitability tested with F waves and cervicomedullary motor evoked potentials (CMEPs).

## Participants and ethics

All experiments were approved by the Human Research Ethics Committee at the University of New South Wales and conformed with the *Declaration of Helsinki*. All participants provided their written, informed consent prior to participating in the study. Participants visited the laboratory on a screening/familiarization day to ensure that responses could be elicited in the muscles of interest by cervicomedullary stimulation and to determine whether the different types of electrical stimulation were tolerated.

During this visit, a medical screening questionnaire was carried out to ensure participant safety and eligibility. Following this initial visit, two individuals were not eligible to participate because CMEPs could not be elicited. In total, 10 healthy individuals participated in the study (age  $33 \pm 12$  years, four females). All participants received remuneration for their time.

### Drug administration

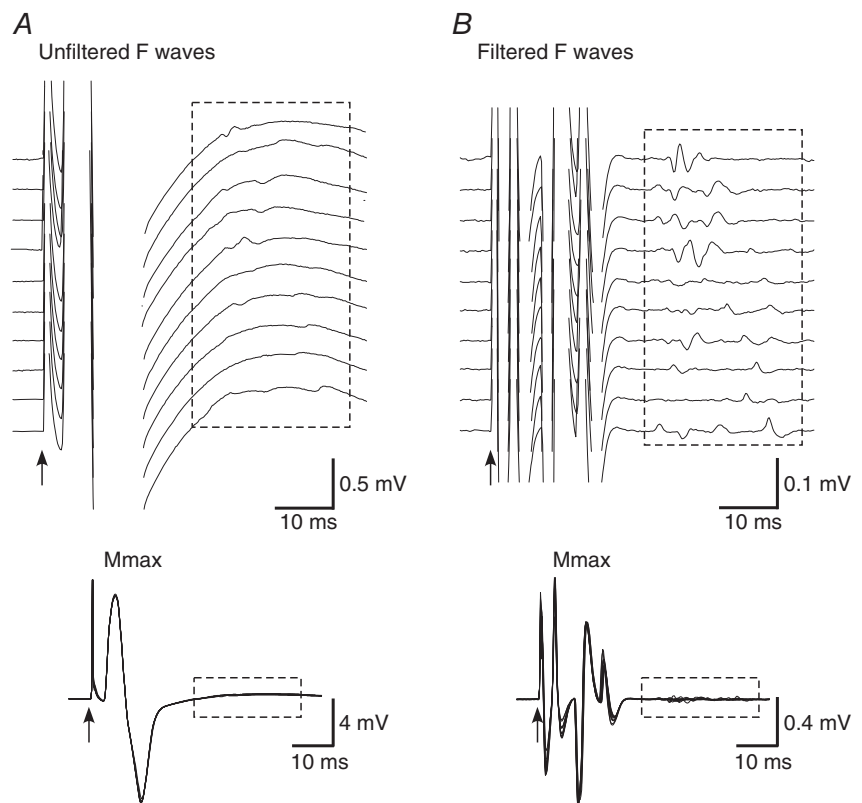
In experimental sessions, participants received either placebo or a 20 mg dose of the anxiolytic drug, buspirone, which was administered orally. The usual therapeutic dose of buspirone is 20–30 mg daily in two to three divided doses (Eison & Temple, 1986). All participants were not taking buspirone or any other medication. The drug and placebo were housed in a capsule to blind participants and the experimenters. The order of drug delivery was randomized by one of the investigators who did not take part in the experimental sessions or initial data analysis. Participants were asked to report any side effects during both visits to the laboratory.

### Set-up

In each session, participants sat with the right arm resting on a table. The hand was pronated and held in place with

Velcro straps across the fingers, the wrist and the forearm. Participants' fingers were loosely taped together to prevent a change in finger position. Additionally, the elbow and proximal forearm were clamped in place by foam blocks to prevent movement of the forearm during brachial plexus stimulation.

Surface electromyography (EMG) recordings were obtained from right biceps brachii (biceps), abductor digiti minimi (ADM) and first dorsal interosseus (FDI) muscles. EMG signals from the biceps were recorded through adhesive Ag-AgCl electrodes (Cleartrace; ConMed Corporation, Utica, NY, USA) placed over the motor point and distal tendon. EMG signals from ADM and FDI were obtained through adhesive EMG Triode electrodes (T3402M; Thought Technology Ltd, Montréal-Ouest, QC, Canada) placed over the muscle belly and the fifth and second metacarpo-phalangeal joints, respectively. EMG signals were amplified ( $\times 300$ ) and bandpass filtered (16–1000 Hz). In addition, highly filtered (200–1000 Hz) and amplified ( $\times 3000$ ) ADM and FDI EMG signals were collected in separate channels for identification and measurement of F waves (Fig. 1) (Khan *et al.* 2012). All EMG signals were processed using CED1902 amplifiers (Cambridge Electronic Design, Cambridge, UK) and sampled at 5 kHz using a 16-bit A/D converter (CED1401) and Spike 2, version 7 (Cambridge Electronic Design).



**Figure 1. Measurement of F waves**

Raw traces of the same ADM F waves collected using 16–1000 Hz filtering (**A**) and 200–1000 Hz filtering (**B**) (highly-filtered). Top: raster of 10 consecutive F-wave traces. Bottom: superimposed traces and maximal M waves. The boxed rectangles encompass the F waves.

## Stimulation

Three types of stimulation were used in the present study and these were all performed at rest. These included stimulation of the corticospinal tract axons at the level of the cervicomedullary junction, stimulation of the brachial plexus and stimulation of the ulnar nerve. All types of stimulation were well tolerated, albeit uncomfortable.

**Cervicomedullary stimulation.** Corticospinal tract axons were stimulated by single electrical pulses (0.2 ms pulse width) delivered by a constant current stimulator (DS7AH; Digitimer, Welwyn Garden City, UK) through two surface electrodes positioned posterior and superior to the tips of the mastoid processes. The cathode was placed on the left to reduce the likelihood of spread of the stimulus to the ventral roots supplying the right biceps. Responses were recorded from the biceps and the stimulus intensity (105–240 mA) was adjusted to evoke a CMEP with an amplitude of ~10% of the maximal M wave.

**Brachial plexus stimulation.** Maximal M waves were evoked in biceps by electrical stimulation of the brachial plexus through surface electrodes positioned in the supraclavicular fossa (Erb's point: cathode) and on the acromion (anode) (DS7AH constant current stimulator; pulse width 0.2 ms). Stimulus intensity was set 20% above the level required to produce a maximal compound muscle action potential ( $M_{\max}$ ) in the biceps (24–160 mA).

**Ulnar nerve stimulation.** To evoke F waves in the ADM, supramaximal electrical stimuli were delivered to the ulnar nerve at the wrist (DS7AH constant current stimulator; pulse width 0.2 ms). Electrodes were placed ~2 cm apart over the nerve with the cathode positioned distal to the anode and ~2 cm proximal to the wrist. Stimulus intensity was set at 20% above the level required to produce a maximal compound muscle action potential in ADM (30–54 mA). Potentials were also recorded from ulnar-innervated FDI, although supramaximal stimulation for this muscle was not ensured. If all motor axons were not stimulated, then the possibility cannot be ruled out that some component of the response at F-wave latency could occur via activation of motoneurons through the synaptic influence of Ia afferent input. During the present study, stimuli were delivered in sets of 30 at a rate of 2 Hz.

## Protocol

Participants were tested on two occasions separated by at least one week. Once the experimental set-up was complete, baseline measurements of F waves, CMEPs and  $M_{\max}$  were obtained. In each measurement set, participants received two blocks of 30 ulnar nerve stimuli (2 Hz) with a 1 min break between blocks. After a brief

break, these were followed by five cervicomedullary stimuli (0.1 Hz) and then two brachial plexus stimuli (0.1 Hz). Up to five baseline measurement sets were performed at 10 min intervals. If CMEPs were not close to the target size, stimulus intensity was adjusted and two or three sets at the new intensity were performed. If CMEPs showed a progressive increase or decrease across sets, a fourth set was performed and the first set discarded from the analysis. After pill intake, measurements resumed at 25 min post intake and again every 10 min up to 105 min post-pill intake. This timing was chosen to ensure that measurements were performed at peak plasma levels of buspirone, which typically occurs between 50 and 90 min following ingestion (Mahmood & Sahajwalla, 1999; Loane & Politis, 2012). By completion of the experiment, eight of the 10 participants had fully recovered from any side effects and motoneurone excitability had recovered.

## Data analysis

All data analysis was performed offline using Signal, version 4 (Cambridge Electronic Design) and Excel (Microsoft Corp., Redmond, WA, USA). Root mean square values were calculated for 100 ms of the EMG signal prior to each stimulus. The areas of the compound muscle action potentials (F waves, M waves and CMEPs) were calculated using built-in software functions. F waves in ADM and FDI were measured from the highly filtered channels (Khan *et al.* 2012). Their areas were first normalized to their corresponding  $M_{\max}$ , also from the highly filtered channel, and then averaged over the 60 sweeps for each time point. F-wave persistence (%) for ADM and FDI was calculated as the percentage of stimuli (out of 60) that evoked an F wave greater than 20  $\mu$ V in amplitude (Khan *et al.* 2012). To determine changes in M-wave areas in ADM and FDI, maximal M-wave areas were measured from the non-highly-filtered channels and averaged over the 60 sweeps in each measurement set. Biceps CMEPs were measured individually, averaged for the five stimuli in each measurement set and then normalized to the average of the two maximal M waves obtained in the biceps during the same set. Pre- and post-drug measurements were normalized to the averaged baseline values and are expressed as a percentage of baseline (mean  $\pm$  SD). F-wave persistence was not normalized and is reported as a raw value (mean  $\pm$  SD). Lastly, differences in measurements between days were calculated by subtracting the buspirone measurements from the placebo measurements at each time point and these are expressed as the mean  $\pm$  95% confidence interval (CI).

## Statistical analysis

Results are presented as the mean  $\pm$  SD in the text and figures. Differences between days are presented as the



mean (95% CI). All statistical analyses were performed with SPSS, version 22 (IBM Corp., Armonk, NY, USA). Two-way repeated measures ANOVAs were carried out to determine the effects of drug and time on the F waves, CMEPs and maximal M waves. Mauchly's test of sphericity was performed and, for data that were not spherical, Greenhouse–Geisser correction was used. When a significant drug  $\times$  time interaction was found, a *post hoc* Bonferroni test was used to determine at which time points buspirone values differed from placebo values. One-way repeated measures ANOVAs were used to determine whether the three baseline measurements differed within days and whether background EMG differed within days. Paired *t* tests were used to determine whether baseline measurements and background EMG differed between days.  $P < 0.05$  was considered statistically significant.

## Results

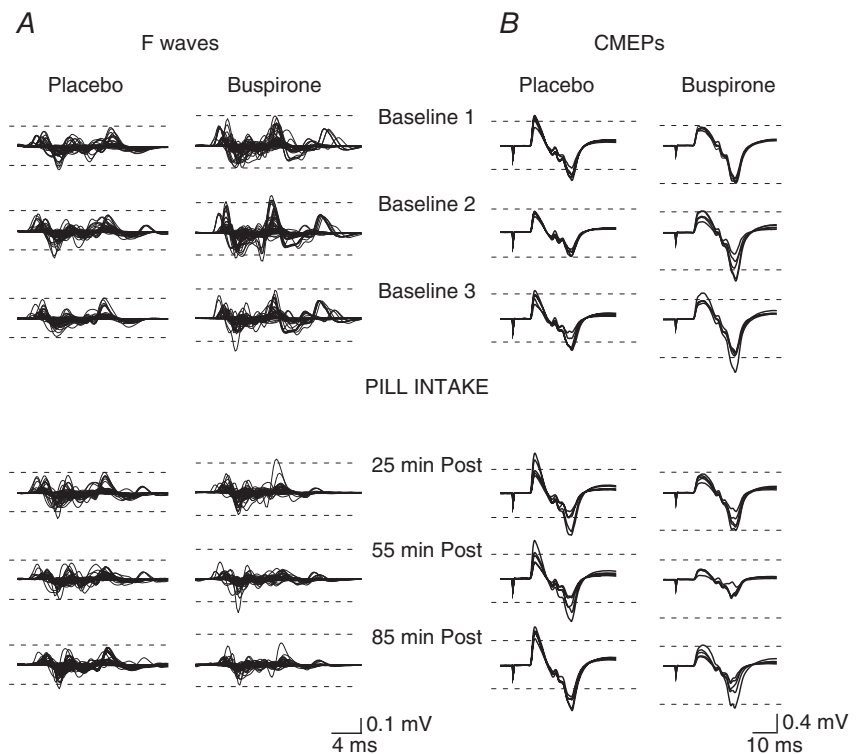
Ingestion of buspirone decreased motoneurone excitability as indicated by reductions in F waves measured in two intrinsic hand muscles and CMEPs measured in biceps brachii. Side effects were reported by eight participants on the buspirone day. These varied between participants and included light-headedness, drowsiness, blurry vision, nausea and sweating. These side effects were short-lasting with recovery within 40 min of

onset and occurred between 35 and 105 min following pill ingestion. Three of these same participants also reported side effects on the placebo day, which included feeling cold, feeling strange and experiencing chills.

## F waves

Raw F-wave traces from a single participant are shown in Fig. 2A. Compared to the placebo, F waves were reduced following buspirone intake.

For the group, the area of F waves in the ADM was  $\sim 7\%$  of the corresponding  $M_{\max}$  during both visits (placebo:  $6.8 \pm 2.6\%$ , buspirone:  $7.1 \pm 2.8\%$ ;  $P = 0.49$ ) and had high persistence (placebo:  $92 \pm 7\%$ , buspirone:  $93 \pm 7\%$ ,  $P = 0.46$ ). Following pill intake, mean F-wave area and persistence were decreased after buspirone ingestion but not placebo (Fig. 3). For F-wave area, a two-way repeated measures ANOVA showed significant effects of drug ( $F_{1,9} = 16.3$ ,  $P = 0.003$ ) and time ( $F_{9,81} = 3.49$ ,  $P = 0.001$ ) and a drug  $\times$  time interaction ( $F_{9,81} = 2.52$ ,  $P = 0.013$ ) with *post hoc* tests showing a significant reduction on the buspirone day compared to the placebo day from 35 to 75 min (Fig. 3A). Persistence of F waves showed drug and time effects (drug:  $F_{1,9} = 7.91$ ,  $P = 0.02$ ; time:  $F_{2,50, 22.22} = 6.82$ ,  $P = 0.003$ ) with no interaction effect ( $F_{2,48, 22.38} = 2.27$ ,  $P = 0.11$ ) (Fig. 3B). The differences in ADM F-wave area and persistence between the buspirone and placebo day for each individual participant are shown



**Figure 2. Individual subject F waves and CMEPs on placebo and buspirone days**

Raw EMG traces of ADM F waves (60 traces overlaid) (A) and biceps CMEPs (five traces overlaid) (B) from the same individual subject on the placebo (left) and buspirone days (right) before and at selected times after pill ingestion. F waves are shown without the preceding M waves. Supramaximal ulnar nerve stimuli were delivered at 22 ms prior to the start of each trace. For CMEPs, stimulus artefacts indicate timing of cervicomedullary stimulation. Dotted lines are drawn to aid comparison between times and encompass the smallest set of potentials recorded at baseline on each day. Both F waves and CMEPs are reduced after ingestion of buspirone.

in Figs 3C and D. The mean difference in ADM F-wave area post pill intake was  $-27\%$  ( $-42, -12$ ) and the mean difference in persistence was  $-9\%$  ( $-16, -2$ ).

Maximal M waves in ADM remained constant throughout both visits and did not differ between days (all  $P > 0.45$ ). Because participants were relaxed throughout the protocol, levels of incidental EMG were very low and were similar between days and throughout days (placebo:  $1.3 \pm 0.5 \mu\text{V}$ , buspirone:  $1.2 \pm 0.4 \mu\text{V}$ , all  $P > 0.23$ ).

F waves recorded from first dorsal interosseous (FDI) displayed a similar pattern to those in ADM with decreases in both the F-wave area and persistence after buspirone intake (not shown).

### CMEPs

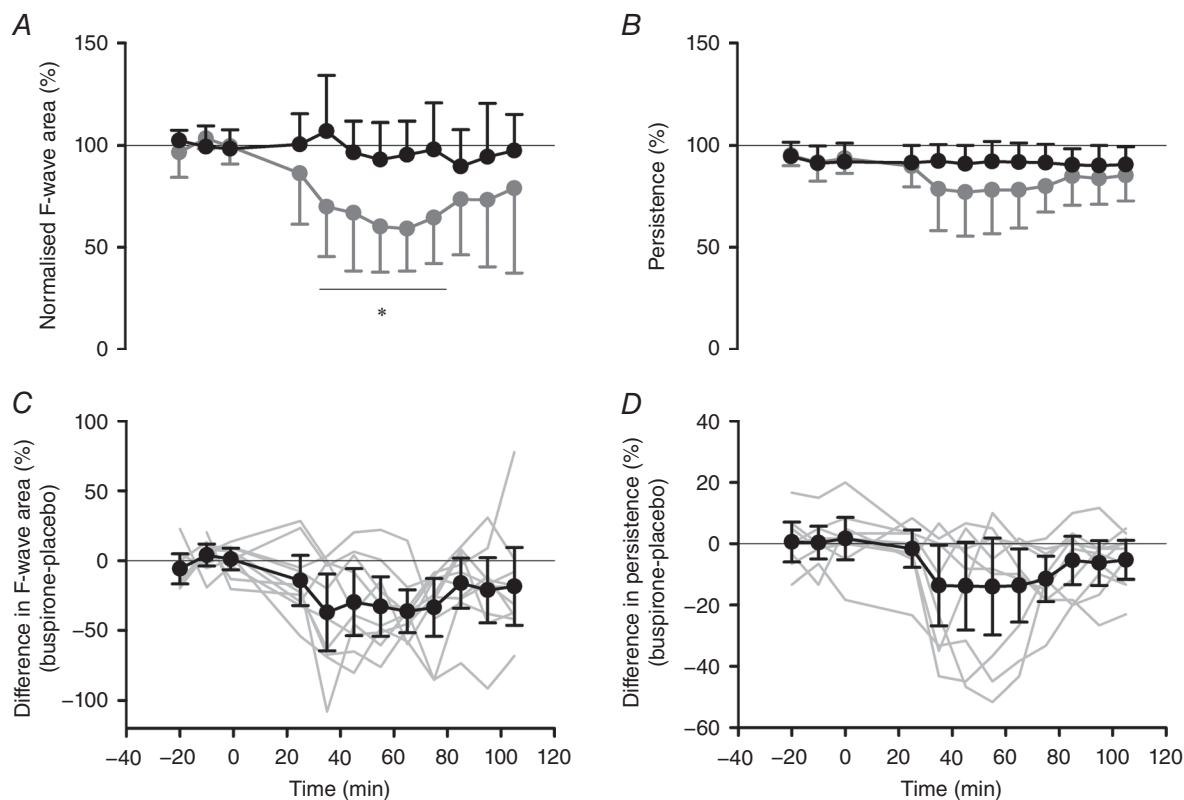
CMEP raw traces recorded from biceps from an individual subject are shown in Fig. 2B. Similar to the F waves in this subject (Fig. 2A), CMEPs declined dramatically following intake of buspirone but not placebo.

For the group, baseline CMEPs were  $12 \pm 6\%$  of maximal biceps M-waves during both visits ( $P = 0.45$ ).

Although normalized CMEP area decreased on both days following pill intake (Fig. 4A), this decrease was greater following buspirone. Two-way repeated measures ANOVA showed a significant drug effect ( $F_{1,9} = 5.86, P = 0.039$ ) but no time effect ( $F_{2,12,19.06} = 2.153, P = 0.14$ ), nor a drug  $\times$  time interaction ( $F_{1,43,12.88} = 1.235, P = 0.31$ ). The time course of differences in CMEP area for individual participants varied (Fig. 4B, grey lines) with the mean difference in CMEP area post pill intake  $-31\%$  ( $-60, -2$ ). Maximal biceps M waves did not differ within days or between days (all  $P > 0.05$ ). Incidental biceps EMG was very low and similar between and within days (placebo:  $2.7 \pm 0.7 \mu\text{V}$ , buspirone:  $3.1 \pm 1.1 \mu\text{V}$ , all  $P > 0.38$ ).

### Discussion

The key novel finding of the present study is that ingestion of a 5-HT<sub>1A</sub> receptor agonist (buspirone) depresses motoneurone excitability in healthy humans. Reduction of motoneurone excitability by activation of 5-HT<sub>1A</sub> receptors is consistent with previous work on the turtle spinal cord (Cotel *et al.* 2013; see Introduction), and



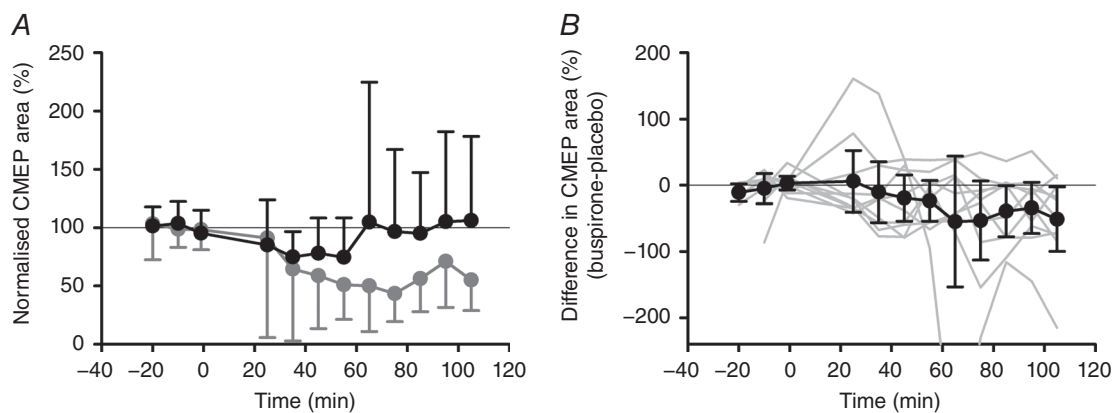
**Figure 3. Changes in ADM F-wave area and persistence following buspirone intake**

Group mean  $\pm$  SD of normalized ADM F-wave area (A) and ADM F-wave persistence (B) on placebo (black circles) and buspirone (grey circles) days. F-wave area was first expressed relative to the area of corresponding M waves and then as a percentage of the mean of the baseline trials on each day. Group differences between buspirone and placebo days ( $\pm 95\%$  CI: black circles) for normalized F-wave area (C) and persistence (D). Grey lines indicate individual subject data. Pill ingestion occurred at time 0. Significant differences for drug  $\times$  time interaction effect identified by *post hoc* tests:  $*P < 0.05$ .

supports the concept that activation of these receptors during fatiguing exercise could reduce motoneurone output.

Two measures were used to assess whether buspirone intake altered motoneurone excitability. Cervicomedullary stimulation activates motoneurons synaptically and has a large monosynaptic component when measured in biceps brachii (Petersen *et al.* 2002; Taylor & Gandevia, 2004). Thus, the decrease in CMEPs following buspirone intake could be explained by decreases in (i) the excitability of spinal motoneurons; (ii) the efficacy of corticomotoneuronal synapses or (iii) the excitability of corticospinal tract axons at the site of stimulation. By contrast, F waves represent recurrent discharge of motoneurons after invasion of their soma by the antidromic action potentials evoked by stimulation of the peripheral nerve (Eccles, 1955; McLeod & Wray, 1966). Because stimulation of the motor axons remained supramaximal throughout the experiments, F-wave size and persistence depended only on the excitability of the motoneurons. In general, although not always, a decrease in recurrent discharges reflects a decrease in motoneurone excitability (Schiller & Stalberg, 1978). Taken together, the decreases in both F waves and CMEPs are a strong indication of decreased motoneurone excitability following buspirone intake. Methodological limitations required us to elicit CMEPs and F waves in different muscles. Discomfort associated with the high stimulus intensities that are required makes it difficult to elicit CMEPs in hand muscles, whereas F waves cannot be measured in proximal muscles because of the short latency of the responses. However, because oral buspirone produced systemic effects, there is no reason to expect different actions on the two motoneurone pools tested in the present study.

We argue that the depression of motoneurone excitability after ingestion of buspirone is a result of the activation of 5-HT<sub>1A</sub> receptors on the motoneurons, probably at the axon initial segment of the motoneurons. First, electrophysiological work carried out in animal models supports a decrease in motoneurone excitability following activation of 5-HT<sub>1A</sub> receptors located on the motoneurons (Jackson & White, 1990; Rasmussen & Aghajanian, 1990; Cotel *et al.* 2013). Recent work on the turtle spinal cord has confirmed that, when 8-OH-DPAT (a 5-HT<sub>1A</sub>/5-HT<sub>7</sub> receptor agonist) is focally applied at the axon initial segment of the motoneurone in the presence of a 5-HT<sub>7</sub> receptor antagonist, action potential generation is inhibited (Cotel *et al.* 2013). Application of high concentrations of 8-OH-DPAT to spinal motoneurons in the rat inhibits glutamate-evoked motoneurone firing, although low concentrations have little or no effect (Jackson & White, 1990). Second, labelling has demonstrated 5-HT<sub>1A</sub> receptors in the ventral horn of the aged human spinal cord with greater numbers of receptors in thoracic and lumbar regions than in cervical regions (Laporte *et al.* 1996). Similar distributions are seen in the rat (Marlier *et al.* 1991). Third, buspirone behaves as a selective partial agonist to the 5-HT<sub>1A</sub> receptors with no affinity for other 5-HT receptors, although it does display some affinity for dopamine (D<sub>2</sub>-like) receptors (McMillen *et al.* 1983; Eison & Temple, 1986; Loane & Politis, 2012). Although mammalian spinal motoneurons express both the D<sub>1</sub>-like and D<sub>2</sub>-like receptors (Han *et al.* 2007; Han & Whelan, 2009; Schwarz & Peever, 2011), to date, most of the effects of dopamine on motoneurone excitability have been attributed to activation of D<sub>1</sub>-like receptors, with no effect of antagonists or agonists to D<sub>2</sub>-like receptors (Han & Whelan, 2009; Schwarz & Peever, 2011; Sharples *et al.* 2014). Thus, the reduction in CMEPs and



**Figure 4. Changes in biceps CMEP area following buspirone intake**

*A*, group mean  $\pm$  SD of normalized biceps CMEP area. CMEP area was expressed relative to the corresponding M wave and then as a percentage of the mean of the baseline trials on each day. *B*, group differences between buspirone and placebo days ( $\pm$  95% CI: black circles) for normalized biceps CMEP area. Grey lines indicate individual subject data. For one subject, one point ( $-432\%$ ) at 65 min is outside the graph. Pill ingestion occurred at time 0.

F waves following buspirone intake is probably mediated by activation of 5-HT<sub>1A</sub> receptors located directly on the motoneurons.

Despite the probable actions of buspirone at the motoneurons, we cannot exclude the possibility that buspirone ingestion may have indirectly affected motoneurone excitability through a change in descending monoaminergic drive to the spinal cord (Sotelo *et al.* 1990; Fornal *et al.* 1994; Azmitia *et al.* 1996; Dalley *et al.* 1996; Gobert *et al.* 1999; Reader *et al.* 2000; Silverstone *et al.* 2012). However, because buspirone and its metabolite have opposing effects on the serotonergic and noradrenergic systems, indirect effects are difficult to predict. Moreover, such effects may be small in the present study because all measurements were made at rest. Although the strong electrical stimuli used to elicit CMEPs and F-waves may produce arousal, studies in behaving cats suggest that nociceptive stimulation does not increase firing of descending serotonergic neurones (Auerbach *et al.* 1985; Jacobs *et al.* 2002). Rather, firing is best associated with motor output (Jacobs *et al.* 2002).

Buspirone has been shown to inhibit the release of 5-HT from the raphe nuclei via 5-HT<sub>1A</sub> autoreceptors (Sotelo *et al.* 1990; Fornal *et al.* 1994; Azmitia *et al.* 1996). By contrast, via the actions of its metabolite on NA- $\alpha$ 2 receptors, it has also been shown to increase the release of noradrenaline from the locus coeruleus (Dalley *et al.* 1996; Gobert *et al.* 1999; Silverstone *et al.* 2012), from which neurones innervate multiple spinal and cortical areas. 5-HT and noradrenaline can alter motoneurone excitability via the 5-HT<sub>2B/C</sub> and noradrenaline NA- $\alpha$ 1 receptors, respectively, located on the motoneurons. Both the serotonergic and noradrenergic receptors are G-q coupled excitatory receptors and increase motoneurone excitability through the activation of large persistent inward currents, as well as by decreasing leak conductances and consequently altering the resting membrane potential of the motoneurons (Perrier & Delgado-Lezama, 2005; Harvey *et al.* 2006; Li *et al.* 2007; D'Amico *et al.* 2013*b*). Any changes in the activation of persistent inward currents would not affect the measurements used in the present study because these slowly-activating currents require long-duration inputs to be fully activated (Murray *et al.* 2011; D'Amico *et al.* 2013*a*) and therefore would not be activated in response to brief electrical stimuli. However, both CMEPs and F waves could be affected by changes in resting membrane potential. Because buspirone has opposing actions on descending serotonergic and noradrenergic drive to the motoneurons, any decrease in excitability as a result of the removal of serotonergic drive to the motoneurons would probably be negated by the concomitant increase in noradrenergic drive to the spinal cord. Additionally, for rat motoneurons, the basal levels of serotonin, as recorded within the serotonergic ventral funiculus in rats at rest (Gerin *et al.*

1995), are considerably lower than the concentrations of 5-HT required to alter motoneurone resting membrane potential and the threshold for action potential spike initiation (Harvey *et al.* 2006). Finally, indirect evidence from humans also suggests that there are low levels of 5-HT and noradrenaline in the spinal cord at rest. Namely, the selective 5-HT reuptake inhibitor citalopram and the selective noradrenaline reuptake inhibitor reboxetine both fail to alter motoneurone excitability tested at rest with F-wave responses (citalopram and reboxetine) and H reflexes (reboxetine) (Plewnia *et al.* 2002; Robol *et al.* 2004). Furthermore, blockade of 5-HT<sub>2</sub> and NA- $\alpha$ 1 receptors with chlorpromazine also has no effect on resting H-reflexes (Metz *et al.* 1982). Measurements in the present study were made at rest and, when taken together with the arguments above, it is difficult to attribute the large depression (~30% decrease in responses) in excitability of spinal motoneurons after buspirone intake to the effects of buspirone on monoaminergic release.

### Could depression of motoneurons through activation of 5-HT<sub>1A</sub> receptors contribute to central fatigue in human voluntary contractions?

Depression of motoneurone excitability and slowing of motoneurone firing rates are commonly reported in association with fatiguing exercise, and are proposed to contribute to central fatigue (Bellemare *et al.* 1983; Bigland-Ritchie *et al.* 1983; Gandevia *et al.* 1999; Butler *et al.* 2003; Khan *et al.* 2012). The findings of the present study are consistent with the proposal that activation of 5-HT<sub>1A</sub> receptors could be a mechanism that contributes to such changes. However, physiological activation of inhibitory 5-HT<sub>1A</sub> receptors may require extrasynaptic spread of 5-HT from the synapses on the soma and dendrites. In the turtle spinal cord, Cotel *et al.* (2013) observed inhibition of motoneurone output only after sustained stimulation of the serotonergic dorsolateral funiculus. Thus, this mechanism probably operates only when serotonergic drive to the motoneurons is high, and it is not known when high serotonergic drive occurs in humans. Studies in intact, freely-behaving animals have shown a link between descending serotonergic drive and motor activity (Fornal *et al.* 1985; Fornal *et al.* 1996; Jacobs & Fornal, 1997; Jacobs *et al.* 2002). In humans, a recent study that examined the effects of serotonin as a spinal gain modulator suggest that 5-HT release is graded with the strength of voluntary contractions, and that this release is quite diffuse (Wei *et al.* 2014). Thus, 5-HT release could be high during strong voluntary contractions in humans and this may explain the parallels between resting motoneurone behaviour during and following 2 min of maximal voluntary contraction in humans and 3 min of stimulation of the dorsolateral funiculus in the turtle spinal cord (McNeil *et al.* 2009; Cotel *et al.* 2013).



The proposition that high serotonergic drive reduces motoneurone excitability runs counter to the idea that motoneurone excitability is enhanced because of high descending monoaminergic drive with stress or arousal (Heckman *et al.* 2003). One possible explanation may be that stress-related descending monoaminergic drive may be more noradrenergic than serotonergic (Auerbach *et al.* 1985; Abercrombie & Jacobs, 1987; Jacobs *et al.* 2002).

In the present study, we have examined the effects of buspirone on motoneurons at rest. It still remains unclear how activation of 5-HT<sub>1A</sub> receptors may affect task performance. A few studies have demonstrated that oral intake of buspirone can impair performance during motor tasks in normal and high ambient temperatures (Marvin *et al.* 1997; Bridge *et al.* 2001; Bridge *et al.* 2003). These effects were attributed without direct evidence to the non-serotonergic effects of buspirone at the supra-spinal level (Bridge *et al.* 2003). However, none of the above studies examined changes at the spinal cord level. Our results indicate that buspirone intake leads to a decrease in motoneurone excitability and this may have contributed to the reduced motor performance noted in previous studies. Specifically, there was an increase in ratings of perceived exertion (Marvin *et al.* 1997) along with a reduction in time to task failure following buspirone intake (Marvin *et al.* 1997; Bridge *et al.* 2001; Bridge *et al.* 2003), which may reflect the increased central drive required to overcome inhibition of the motoneurons. It would be ideal to determine whether administration of a 5-HT<sub>1A</sub> receptor antagonist reduces central fatigue during motor performance; however, a suitable 5-HT<sub>1A</sub> receptor antagonist for human use is not yet available. Therefore, further studies are required to determine whether the effects of buspirone on motor performance are in part mediated via its actions at the spinal level.

To conclude, in individuals at rest, ingestion of a selective 5-HT<sub>1A</sub> receptor partial agonist reduces motoneurone excitability. This reduction is consistent with the activation of inhibitory 5-HT<sub>1A</sub> receptors on the axon initial segment of the motoneurons and the proposal that this mechanism could contribute to the reduction in motoneurone output during fatiguing exercise.

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## Additional information

### Competing interests

The authors declare that they have no competing interests.

### Author contributions

This work was carried out at Neuroscience Research Australia, in Sydney, Australia. JMD, AAB, MEH, FC, JFMP, JEB, SCG and JLT contributed to the conception and design of the study. JMD and AAB collected the data. JMD analysed the data. JMD, AAB, MEH, JEB, SCG and JLT were involved in interpretation of the analysed data. JMD and JLT drafted the manuscript. All authors approved the final version of the manuscript and all persons who qualify for authorship are listed.

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