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Effect of standing posture on inhibitory postsynaptic potentials in gastrocnemius motoneurons

SJ Garland[1], A Gallina[2], CL Pollock[2], TD Ivanova[1]

[1]Department of Physical Therapy and [2]Graduate Program in Rehabilitation Sciences, University of British Columbia, Vancouver, British Columbia, Canada

Running Head: Gastrocnemius motoneuron inhibition in standing

Corresponding Author:
S. Jayne Garland, PhD PT
Professor and Dean,
Faculty of Health Sciences
Western University
1151 Richmond St.
Arthur and Sonia Labatt Health Sciences Bldg., Room 200J
London, ON Canada N6J 5B9
Phone: (+1) 519 661 4239
Email: jgarland@uwo.ca

Abstract

This study examined the task-dependency of sensory inputs on motoneuron excitability by comparing the inhibitory post-synaptic potential (IPSP) evoked by stimulation of the sural nerve between a standing postural task (Free Standing) and a comparable voluntary isometric contraction performed in supine (Lying Supine). We hypothesized that there would be a smaller IPSP in standing than in supine, based on the task dependence of the ankle plantarflexor activity to the standing task. Ten healthy participants participated in a total of 15 experiments. Single motor unit (MU) firings were recorded using both intramuscular fine wire electrodes and high density surface electromyography (HDsEMG). Participants maintained the MU discharge at 6-8 Hz in Free Standing or Lying Supine while the right sural nerve was stimulated at random intervals between 1 and 3 s. To evaluate the reflex response, the firing times of the discriminated motor units were used to construct peri-stimulus time histograms (PSTH) and peri-stimulus frequencygrams (PSF). The sural nerve stimulation resulted in weaker inhibition in Free Standing than Lying Supine. This finding is discussed in relation to the putative activation of persistent inward currents in standing posture and the task-dependent advantages to overriding inhibitory synaptic inputs to the plantarflexors to maintain the standing posture.

New & noteworthy

The task-dependent modulation of sensory inputs on motoneuron excitability in standing is not well understood. Evoking an Inhibitory Post Synaptic Potential (IPSP) resulted in a smaller IPSP in gastrocnemius motoneurons in standing than in supine. Mildly painful sensory inputs produced weaker motoneuron inhibition in standing, suggesting an imperative to maintain ankle plantarflexion activity for the task of upright stance.

Keywords

Motor unit, postsynaptic inhibition, standing posture, peri-stimulus time histogram

Introduction

Standing posture involves bilateral activation of the ankle plantarflexor musculature. According to the 'inverted pendulum' model of standing posture (Winter et al. 1998), the central nervous system adjusts the center of pressure (COP) to maintain upright stance through activation of soleus (Masani et al. 2003) and gastrocnemius (Gatev et al. 1999; Masani et al. 2003) muscles. Previous work in our laboratory investigating the control of motor units in quiet stance revealed a significant amount of common modulation of motor units in soleus (Mochizuki et al. 2006). Possible sources of the common drive in standing included ionotropic inputs, generated by sensory inputs and descending commands, such as proprioceptive (Mochizuki et al. 2007) and vestibulospinal (Monsour et al. 2012) inputs.

Evidence is mounting for the participation of the cortex in postural control whereby sensorimotor responses to postural perturbation are adapted in a task-dependent manner (Jacobs and Horak 2007). There is also considerable evidence supporting cortical involvement in sensory gating during posture (Saradjian 2015). Sensory gating, a process whereby the inflow of somatosensory information is suppressed, is prevalent in movement, possibly serving to reduce redundant information from reaching the cortex (Song and Francis 2015). Movement-related sensory gating *at the spinal level* is context dependent (Confais et al. 2017). Less is known about the presence of sensory gating in the spinal cord during postural tasks.

We examined the task-dependent modulation of gastrocnemius motoneuron excitability in standing versus lying supine. To do so, we compared the inhibitory post-synaptic potential (IPSP) evoked by stimulation of the sural nerve between a standing postural task and a comparable voluntary isometric contraction performed while lying supine. We hypothesized that the task of standing would result in a smaller IPSP in standing than in supine.

Methods

2.1 Participants

Ten healthy participants (aged 22-56 years; 4 female) with no known neuromuscular disorders participated in a total of 15 experiments after providing informed written consent. All experimental procedures were approved by the University of British Columbia Clinical Research Ethics Board and conformed to the standards established by the Declaration of Helsinki (2008).

2.2 Electrical stimulation and Electromyography (EMG)

Sural nerve stimulation has evoked a robust IPSP in gastrocnemius in past studies (Brooke et al. 1997; Khan and Burne 2010; Rogasch et al. 2012). In this study, the sural nerve of the right foot was stimulated through bipolar electrodes (1 cm^2, 3 cm apart) positioned below the lateral malleolus over the sural nerve trunk. Single square pulse stimuli of 500μs duration were delivered by a constant-current stimulator DS7 (Digitimer Ltd., Hertfordshire, UK) triggered through Power 1401 with Spike 2 software (Cambridge Electronic Design Ltd., Cambridge, UK). Perceptual threshold (PT) was determined by increasing the stimulator intensity in 1 mA increments until the participant reported sensation. Stimulator intensity was then reduced until the participant reported no sensation; the last intensity that the participant could perceive was taken as threshold. Reflex stimulation intensity was set at 7 times PT; at this intensity, subjects reported mild pain sensation (3 out of 10).

Single motor unit firings were recorded using both intramuscular fine wire electrodes and high density surface electromyography (HDsEMG). Fine wire electrodes consisted of three insulated Teflon-coated stainless steel wires (50 μm diameter, California Fine Wire Company, CA, USA) bonded together and passed through a 25-gauge hypodermic needle (Becton Dickinson, Franklin Lakes, NJ, USA). A small hook at the terminal end of the fine wire electrode held the electrode in place after the needle was removed. The exposed tips of two out of 3 wires formed a bipolar electrode that recorded motor unit action potentials from the medial gastrocnemius muscle. The third wire allowed the freedom to configure the bipolar electrode differently, should the first configuration yield an undesirable signal.

The signal was bandpass filtered (10-10,000 Hz), differentially amplified (CMMR > 90 dB at 60 Hz input impedance 10 MΩ, Coulbourn Instruments, PA, USA) and sampled at 25,000 Hz. Single MU action potentials were discriminated on-line using Spike 2 software with a template matching algorithm.

The HDsEMG grid (semi-disposable adhesive matrix; OTBioelettronica, Torino, Italy) consisted of 64 electrodes spaced 8 mm apart, arranged in 5 columns and 13 rows (an electrode missing in one of the corners). Electromyographic signals were collected in monopolar modality using a HDsEMG amplifier (128-channel EMG-USB; OTBioelettronica, Torino, Italy). Signals were amplified 2000 times, sampled at 2048 Hz and stored for off-line motor unit action potential extraction. The positions of the fine wire electrode in the right medial gastrocnemius muscle and the HDsEMG grid are depicted in Figure 1A.

2.3 Experimental Protocol

Participants were positioned on a tilt table, a standing frame that is used in clinical practice to enable supported stance, which allowed us to move the participant easily between standing and supine with minimal changes in body position. A force platform (AccuGait, Advanced Mechanical Technology, Watertown, MA, USA) was secured to the base of the tilt table to measure either 1) the plantarflexion forces exerted by an isometric contraction in supine or 2) the postural sway in standing.

Two conditions were tested in each experiment: Free Standing and Lying Supine. The order of the testing conditions (Free Standing or Lying Supine) was randomized. In the Free Standing condition, the tilt table behind the participant was vertical with the foot support parallel to the floor (Figure 1B). In the Lying Supine condition, the tilt table supporting the participant was horizontal and the foot support was vertical (Figure 1C). Before the tilt table was transitioned from Free Standing to Lying Supine, supports were placed between the participant and the table to avoid a change in body position (Figure 1C). If Lying Supine was performed first, the straps and supports were removed for Free Standing condition after transitioning slowly from supine to standing.

Before the experiment started, the participant stood in a comfortable position to determine if the intramuscular electrode was collecting single MUs in standing. Intramuscular MUs were more difficult to isolate in standing than supine and we wanted to ensure that MU activity could be observed in both conditions before starting the experiment. The single MU was discriminated on-line using template matching algorithm (Spike 2 v.6, Cambridge Electronic Design, Cambridge, UK) and the acceptance pulses were displayed for the participant as instantaneous firing frequency on a screen. The audio signal associated with the acceptance pulses served as auditory feedback of MU discharge. Once we were sure of the quality of the intramuscular MU recording, the experiment began according to the randomized order of conditions.

In the Free Standing condition, participants stood on the force platform without touching the tilt table which allowed for natural body sway. In the Lying Supine condition, ankle plantarflexion results in movement of the body along the tilt table. To prevent this movement, the participant's heels were placed on a rigid support and non-compliant straps, which were attached between the foot support of the tilt table and a belt on participant's waist, maintained the position of the legs and feet similar to that in standing. In Lying Supine, participants were asked to produce isometric contractions of the plantarflexors of the right leg (the side that was stimulated). Participants performed two ramp-and-hold voluntary ankle plantarflexion contractions in Lying Supine; the force was gradually increased until motor units were recruited in the intramuscular recording, held for 5 seconds and then gradually lowered. The force associated with the first firing of the MU was deemed the Recruitment Threshold. Subsequently, a low-force contraction sufficient to recruit a motor unit on the intramuscular wire was performed. Participants tried to maintain the motor unit on the intramuscular wire that was active in the first testing condition throughout the transition between conditions and during the second task.

In both conditions, participants maintained the MU discharge at 6-8 Hz (in standing or a low force isometric contraction) while the right sural nerve was stimulated at random intervals between 1

and 3 s (see *Electrical stimulation and single motor unit recordings*). On average, 380 stimuli were delivered (minimum of 300; maximum of 500) that were used to evaluate the reflex response. At the end of each experiment, participants performed 2 maximal voluntary contractions (MVCs) in Lying Supine by pressing as hard as they could against the force platform. If the 2 MVCs were not consistent, participants performed a third one. The peak force from all contractions was taken as MVC.

To assess the effect of standing without postural sway, a subset of 4 participants returned on a separate day to repeat the experiment. In these experiments, rather than Free Standing, participants remained strapped onto the tilt-table in standing (Supported Standing condition) to remove the postural sway component from the standing task.

2.4. Kinetic and kinematic data.

Reflective markers were affixed to allow for motion capture of the ankle and knee joints bilaterally (Figure 1). Ten high-speed digital cameras (Motion Analysis Corp, Santa Rosa, CA) sampled the movement of the reflective markers at 100 Hz. Kinematic and kinetic data were analyzed using a custom-written program in post-processing software (Matlab, Mathworks Inc., Natick, MA). The calculated angles were within 0.51° of known angles collected with markers on a goniometer.

Kinetic data were collected using force platform (AccuGait, Advanced Mechanical Technologies Inc., Watertown, MA) sampled at 1000 Hz. Anterior-posterior centre of pressure (APCOP) displacement was calculated in the standing condition. Reflective markers affixed to the force platform ensured that calculations of APCOP were relative to foot and ankle position of participants.

2.4. Data analysis

Identification of motor unit action potentials from the intramuscular EMG was repeated off-line using the same template-matching algorithm (Spike 2) on a file where recordings from both conditions were spliced together. The classified MU action potentials were inspected manually to resolve

interpolation issues. The firing characteristics of the MUs were evaluated by calculating the mean interspike interval (ISI) for the 1 s period immediately before each stimulus. Epochs from interstimulus intervals smaller than 1.5 s and with less than 4 ISIs were not included into the mean calculation.

For HDsEMG recordings, the single MU action potentials were obtained by decomposition of the EMG signal using DEMUSE software (Holobar and Zazula 2007). Motor unit firing rate (Holobar et al. 2010) and reflex inhibition/facilitation (Yavuz et al. 2015) estimated using this method were shown to be valid when compared to gold-standard intramuscular recordings. To identify the MUs that were active in both conditions, epochs from the recordings during each condition were spliced together and decomposed as a single recording and a procedure based on the spatial representation of the motor unit action potential (Dideriksen et al. 2016) was used to verify the correct matching of motor units. Using spike-triggered averaging, the spatial representation of the action potential of each motor unit was obtained for Free Standing and Lying Supine conditions separately. The channels with amplitude higher than 70% of the peak amplitude were identified (Vieira et al. 2010), and the median value of their proximal-distal coordinate was considered to represent the motor unit position. The properties of the action potential identified in both testing conditions were assessed by comparing its spatial representation by calculating the R value of the 2D correlation between average rectified value (ARV) map of the MU action potential in Free Standing vs. Lying Supine condition. As a first step, motor units identified from intramuscular and HDsEMG electrodes were analyzed separately. Motor units were pooled together for further analyses as there was no difference in their firing behaviour between the recording methods.

To assess medial gastrocnemius activation, single differential signals were calculated from the monopolar recordings along the columns of the HDsEMG (now 12x5 channels). The differential signals were then filtered with a band-pass filter (Butterworth, 4^{th} order, 10 – 400 Hz) and full-wave rectified. Epochs of 250 ms prior to the stimulus were extracted from each differential signal and averaged across

143 the channels. Average rectified amplitude of all the channels of the grid was calculated subsequently as
144 the mean of the 250 ms to represent the global surface EMG for the medial gastrocnemius muscle as a
145 whole. The mean value across participants was compared between conditions.
146 To evaluate the reflex response, the firing times of the discriminated motor units were used to
147 construct peri-stimulus time histograms (PSTH) and peri-stimulus frequencygrams (PSF) with a bin size
148 of 0.5 ms around the time of the stimulus (± 250ms). For both PSTH and PSF histograms, the value of
149 each bin was normalized with the average prestimulus bin value (calculated from -250 ms to 0 ms).
150 PSTH and PSF cumulative sums (CUSUMs) were then constructed from the normalized data (Ellaway
151 1978). From the prestimulus period of each CUSUM, maximum and minimum deflections from the
152 prestimulus average were obtained. The larger of the two CUSUM values was then used to make a
153 symmetrical "error box" (Türker et al. 1997). Significant changes in the MU firing following the stimulus
154 were determined by comparing deflections in the CUSUM with the "error box," with deflections in the
155 CUSUM greater in size than the "error box" considered a significant reflex response (Türker and Powers
156 2003). If such large deflections are up-going they were classified as 'excitation' and if they were down-
157 going as 'inhibition'.
158 The inhibitory reflex parameters were measured using the combined PSTH/PSF method
159 (Rogasch et al. 2011; Türker and Powers 2003). The inhibitory reflex latency was taken as the time
160 between 0 ms (initiation of the stimulus) and first turning point of significant PSTH CUSUM, as it better
161 represents the latency of the very first reflex (Todd et al. 2012). Similarly, the end point of the reflex
162 was determined as the second turning point of significant PSF CUSUM. Following the recommendations
163 of Rogasch et al. (2011) and Todd et al. (2012) the duration of the inhibition was calculated as the time
164 between the latency (determined by PSTH method) and the endpoint of the reflex (determined by PSF
165 method). The amplitude of the reflex was determined as the vertical size of the PSTH CUSUM between
166 the first and second turning points divided by the number of stimuli used for that experiment. This value

was then normalized to the maximal possible inhibition (i.e., no spikes in any of the bins throughout the duration of the reflex) calculated using the formula (Brinkworth and Türker 2003):

$$100\% \text{ reflex amplitude} = (k \times \text{reflex duration in bins}) / \text{number of stimuli},$$

where, k is the average prestimulus bin value.

This approach provides the strength of the reflex responses independent to the number of stimuli used and the duration of the reflex. The strength of the reflex is presented as negative number indicating inhibition.

2.5. Statistical analysis.

After determining significant reflex responses using the error box approach, further statistical analysis was performed only on the significant responses. Data from Free Standing / Lying Supine experiments were analyzed separately from the data obtained during the subset of experiments with Supported Standing / Lying Supine. Paired t-tests were used to compare the stimulus intensity and perceived pain as well as the global HDsEMG and joint positions of the ankle and knee between Free Standing (or Supported Standing) and Lying Supine conditions. For single MU analysis, the comparisons of the mean ISI, reflex strength, latency and duration (estimated by PSTH and PSF methods) between Free Standing (or Supported Standing) and Lying Supine were performed using independent Student's t-test when all MUs were considered. For analysis of MUs that were active in both conditions paired t-tests were used.

A secondary analysis was performed to evaluate the influence of motor unit firing rate on reflex strength. A subgroup of motor units with comparable firing rates in both Free Standing and Lying Supine tasks (or Supported Standing and Lying Supine tasks) was selected. For each experiment, the mean ISI of motor units from both tasks were compared and assembled in pairs or small groups having a mean ISI difference of less than 5 ms (approximately 0.5 Hz difference in firing rate). Student's t-tests were used to compare reflex strength and mean ISI of selected MUs between tasks.

The relationships between IPSP amplitude and mean ISI and IPSP amplitude and motor unit recruitment threshold (RT) were assessed by calculating the lines of best fit. The level of significance was set at 0.05. The data are presented as mean ± SD.

Results

The medial gastrocnemius activation was similar in both conditions (Free Standing: 13.5 ± 3.5 μV and Lying Supine: 13.4 ± 6.3 μV; N=15; P=0.94 or Supported Standing: 16.1 ± 11.0 μV and Lying Supine: 15.1 ± 7.4 μV; N=4; P=0.63) The stimulus intensity (Free Standing: 40.2 ± 6.4 mA and Lying Supine: 40.5 ± 7.4 mA; N=15; P=0.70 or Supported Standing: 45.7 ± 8.9 mA and Lying Supine: 45.6 ± 8.8 mA; N=4; P=0.59) and pain ratings (Free Standing: 3.2 ± 0.8 and Lying Supine: 3.5 ± 1.1 out of 10; N=15; P=0.19 or Supported Standing: 3.5 ± 1.3 and Lying Supine: 3.3 ± 1.3 out of 10; N=4; P=0.39) were similar in both conditions.

Before the successful development of various surface electromyography decomposition techniques, intramuscular recordings (needle or fine wire) were the only means to collect single motor unit potential trains and, as such, are considered a "gold" standard in motor unit research. We analyzed the behaviour of the motor units collected with intramuscular electrodes as a way of validating the responses observed in MUs decomposed from the HDsEMG signals.

The motor unit firing characteristics were similar between the intramuscular and HDsEMG recordings (Table 2). Whereas only 1 or 2 motor units were collected per person on the intramuscular fine wire electrodes, an average of 7 ± 4 motor units were decomposed from the HDsEMG per condition per person. There was no difference in the mean ISI in both conditions (Table 2). The coefficient of variation (CV) of the ISI in the Free Standing condition (27.3 ± 7.9%) however was significantly larger ($P<0.001$) than in Lying Supine (21.1 ± 5.4%), despite the same firing rate (see also Table 2). The motor units that were active in both conditions had a high median correlation coefficient of the ARV map of

the MU action potential between Free Standing and Lying Supine (0.94; 25^{th} – 75^{th} percentiles: 0.89-0.96), confirming that the same motor unit was being recorded in both conditions.

The mean number of sural nerve stimuli delivered was 380 ± 75, with a minimum of 300 and a maximum of 500 stimuli per condition per experiment. The sural nerve stimuli resulted in significant inhibition in the majority of motor units recorded with both the intramuscular fine wire and the HDsEMG (84% of the 220 single MUs identified in Free Standing and Lying Supine conditions; also Table 1). Figure 2 depicts PSTH and CUSUM (top) and PSF and CUSUM (bottom) for a single motor unit decomposed from the HDsEMG in Free Standing (left) and Lying Supine (right). Both the PSTH and the PSF CUSUMs reveal clear inhibition in both conditions, with the Lying Supine condition having stronger inhibition than the Free Standing. Both motor unit recording methods (intramuscular and HDsEMG decomposition) rendered the same results when the Free Standing and Lying Supine conditions were compared (Table 2). Across all the MUs, the latency and duration of the reflex were not significantly different between conditions but the strength of inhibition for Lying Supine was greater than in Free Standing (-42.7 ± 24.0% and -56.0 ± 27.7% for Free Standing and Lying Supine, respectively, $P<0.001$).

To explore possible explanations for this finding, we sought to determine if differences in motor unit firing rate between the two conditions might influence the results. There was no significant relationship between the strength of the inhibition and the mean ISI for all motor units, during both, Free Standing and Lying Supine, conditions ($r = -0.09$; $P = 0.3$ and $r = -0.06$; $P = 0.5$, respectively; Figure 3 top). To eliminate the possibility that small differences in firing rate could affect the strength of the inhibition, for motor units that were active in both conditions we plotted the difference in IPSP amplitude against the mean ISI difference between Lying Supine and Free Standing conditions (Figure 3 bottom). Figure 3 shows that the strength of inhibition was unaffected by ISI ($r = 0.03$, $P = 0.8$). Whether the mean ISI was within 10ms between conditions (shaded region Fig 3 bottom), higher or lower, the IPSP was larger in Lying Supine by a comparable amount. Similar results were obtained when

239 the strength of inhibition was compared in a subgroup of motor units with virtually identical firing rates
240 in both tasks (Table 4). For both Free Standing / Lying Supine and Supported Standing / Lying Supine
241 experiments the IPSP was larger in Lying Supine task.
242 We also compared the mean joint angles of the ankle and knee between Free Standing and
243 Lying Supine conditions to determine if body position affected the results. While there was no difference
244 in the knee angle between conditions, the ankle joint angle was 11.2 ± 4.6 degrees more plantarflexed in
245 the Lying Supine than the Free Standing condition (Table 3). The postural sway also introduced a larger
246 CV of the ISI in Free Standing compared to Lying Supine. We, therefore, performed an additional
247 experiment with 4 of the original participants. In this case, the participants repeated the experiment in
248 the opposite order, with the postural sway component removed (Supported Standing). Eliminating the
249 postural sway, the difference in the ankle joint angles between Supported Standing and Lying Supine
250 was only 3.6 ± 1.8 degrees and the CV of ISI was comparable (19.5 ± 4.8% and 17.8 ± 2.9% for Supported
251 Standing and Lying Supine, respectively, P=0.14; also Table 5). The pattern of reflex response was the
252 same in Supported Standing as the Free Standing condition, in that Lying Supine had significantly larger
253 IPSPs than Supported Standing (-58.6 ± 30.3% and -39.7 ± 20.7% for Lying Supine and Supported
254 Standing, respectively; P=0.02; also Table 5).
255 In the Lying Supine condition, the strength of the IPSP was associated with the MU recruitment
256 threshold such that the IPSP amplitude was less in the earliest recruited motor units than the later
257 recruited motor units. This is seen in Figure 4 A where a ramp and hold contraction from a single
258 subject is shown with the firing times of the recruited motor units. It was also found that most motor
259 units were identified from the distal electrodes of the grid (Figure 4 B, lower leg schematic; median
260 position: 10, 25^{th} – 75^{th} percentiles: 8-11.25). For all motor units with an identifiable recruitment
261 threshold (54 out of 103 MUs during Lying Supine condition) there was a moderate correlation (r = -

0.37; P=0.005) between the strength of the inhibition (negative number to indicate inhibition) and the recruitment threshold (Figure 4 C).

Discussion

This study has demonstrated that stimulation of the cutaneous sural nerve evoked less inhibition in the standing position than the supine position, potentially reflecting a task-dependence of the influence of cutaneous sensory inputs onto the motoneuron.

One concept in motor control, as reviewed by Prochazka (1989), is that "the goal of a motor act crucially determines its planning and performance" (p 301). The task-dependency of postural responses to perturbations is well known. In the seminal study by Nashner (1976), adaptive changes to the muscle activation associated with postural perturbations were found on the basis of whether the response would be useful or not to maintain postural stability. Considerable research has been performed over the last decade or two to uncover the cortical and subcortical mechanisms involved in sensorimotor modulation in posture and locomotion. Altenmuller et al. (1995) showed modulation of the somatosensory evoked potentials produced by sural nerve stimulation between stance and different phases of gait. Saradjian (2015) suggested that the central modulation of sensory input is evidence that the central nervous system can modify incoming information based on its relevance to the task. This study adds to this body of literature by showing that sensory inputs that inhibit the motoneurons in supine produce less inhibition in stance, a task that requires ankle plantarflexion activity not only to maintain the MU firing but to maintain upright standing.

The lower IPSP amplitude in standing to same mildly painful cutaneous stimulation as in supine suggests that sensory inputs are gated in standing. Even when the postural sway component of standing was eliminated (supported standing condition), the pattern for a smaller IPSP in supported standing than supine remained. This suggests that vestibulospinal inputs may be involved. Differences in the

sources of central drive to the motoneuron during standing vs. supine may influence the strength of inhibition. Mochizuki et al. (2006) found differences in common drive in the soleus motor units between standing and sitting, suggesting more common drive when standing versus performing an isometric voluntary contraction in sitting. While anecdotal, it is worth commenting on the difficulty some participants had in maintaining the same motor unit discharging on the intramuscular wire between conditions despite minimal change in the body position and the audio feedback of the discharge of the motor units in the transition between conditions. This suggests that different sources of central drive, e.g. vestibulospinal inputs (Grillner et al. 1970), may influence the recruitment of a single motor unit.

Brainstem-derived neuromodulatory inputs, produce dendritic persistent inward currents (PICs) which control the state of excitability of the motoneuron (Heckmann et al. 2005). Persistent inward currents have been theorized to be functionally useful in postural activities such as stance to promote self-sustained firing of motoneurons (ElBasiouny et al. 2010). In mammalian models, the PIC renders the motoneuron less sensitive to excitatory inputs and highly sensitive to inhibitory inputs (Heckman and Enoka 2012). While standing, excitatory inputs to the human ankle plantarflexors imposed by external perturbations resulted in only modest increases in motor unit discharge rate (Pollock et al. 2014); a finding consistent with the presence of a PIC in standing that would reduce the response of the active motor units to excitatory inputs. In a recent study by Revill and Fuglevand (2017), the high sensitivity of the PIC to synaptic inhibition was exploited to blunt the steep increase in motor unit firing rate upon recruitment, suggesting that the presence of a PIC contributes to the non-linear firing rate increases during ascending ramp contractions. Because PICs are highly sensitive to synaptic inhibition, one might have expected a larger IPSP in standing than in supine. Instead, we found that the same sural nerve stimulation resulted in weaker inhibition in standing than supine.

We do not think the relatively flexed ankle joint angle in standing was the explanation for the smaller IPSP amplitude in Free Standing because the IPSP was less in Supported Standing than Lying

Supine when the ankle joint angles were similar. However, in standing, reciprocal inhibition is present and is modulated by concurrent facilitatory corticospinal inputs (Hanna-Boutros et al. 2015). We were not able to measure the amount of reciprocal inhibition between the Standing and Lying Supine conditions so we are unable to speculate on the potentially complex interactions between synaptic and neuromodulatory inputs at the segmental level between these conditions.

We tried to keep the overall level of central drive similar between conditions by asking participants to control the discharge rate of the intramuscularly-recorded motor unit between 6-8 Hz (ISI 125-166ms). On average, the motor unit ISI recorded from the HDsEMG grid was slightly faster than 8 Hz – an ISI of around 120ms in both Free Standing and Lying Supine for all units. Overall the motor units recorded during Lying Supine were not significantly slower than the Supported Standing condition and Figure 3 shows no relationship between firing rate and IPSP strength from both intramuscular and HDsEMG recordings. This, along with the additional analysis in Table 4, suggests that IPSP strength is not solely a function of motor unit discharge rate.

In human experiments, we only have a proxy of central drive in the recordings of net motor unit discharge rate. In mammalian preparations, Berg et al. (2007) showed a balance of excitatory and inhibitory inputs in spinal motoneurons; that is, as excitation was increased, so was inhibition. Therefore it is possible that similar motor unit firing rates in both Standing and Supine conditions are due to increased excitation in standing accompanied by increased inhibition. In cortical neurons, neurons experiencing greater synaptic activation were in a higher conductance state (Bernander et al. 1991; Destexhe et al. 2003). This high conductance state could result in shunting of additional inhibitory currents presented by the sural nerve stimulation in the Standing conditions that was not seen in Lying Supine.

The observation of a larger IPSP amplitude in the earliest recruited motor units is consistent with the finding of larger IPSP in slow twitch versus fast twitch motoneurons (Burke et al. 1970), albeit

all motor units would be considered to be of low threshold in the current experiment. Although we could not compare the Recruitment Thresholds between Standing and Lying Supine conditions, the difference in IPSP strength between standing and supine was observed in the same motor units and therefore the lower IPSP amplitude in standing could not be attributed to a sampling bias.

The main finding of this study was that the strength of inhibitory post-synaptic potentials in motor units of the gastrocnemius evoked by stimulation of the sural nerve was less in a standing postural task than in a supine position. Our data reveal that there is less sensitivity to synaptic inhibition in standing than supine. While speculative, it may be more advantageous to override inhibitory synaptic inputs to the plantarflexors to maintain the standing posture.

Acknowledgements

Current address for SJ Garland and TD Ivanova:

Faculty of Health Sciences, Western University
1151 Richmond St., Arthur and Sonia Labatt Health Sciences Bldg.
London, ON N6J 5B9, Canada

Current address for CL Pollock:

Department of Biomedical Physiology and Kinesiology
Simon Fraser University
8888 University Dr.
Burnaby, BC V5A 1S6, Canada

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Author Contributions

All authors contributed to the conception and design of the research; AG, TDI and CLP performed the experiments and analyzed the data; SJG interpreted the results and drafted the manuscript; all authors discussed and approved the final version of the manuscript.

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Tables

Table 1. Number of motor units identified in Free Standing and Lying Supine conditions

Motor Units	All			Active in both conditions		
	Total number	Number with Inhibition	Inhibition present (% total)	Total number	Number with Inhibition	Inhibition present (% total)
Intramuscular Free Standing	17	15	88	9	7	78
Intramuscular Lying Supine	17	15	88	9	8	89
HDsEMG Free Standing	99	76	77	27	17	63
HDsEMG Lying Supine	123	103	84	27	22	81

HDsEMG – high density surface electromyogram.

Table 2. Reflex parameters and MU firing characteristics during Free Standing and Lying Supine

Parameter	Motor Units	Intramuscular Free Standing	Intramuscular Lying Supine	HDsEMG Free Standing	HDsEMG Lying Supine
Reflex Amplitude (% max amplitude) *from PSTH CUSUM*	All	-53.1 ± 26.9	-66.9 ± 37.3^{+}	-40.0 ± 23.3	-54.4 ± 25.8*
	Active in both conditions	-47.3 ± 36.8	-57.0 ± 32.7*	-32.8 ± 13.9	-54.6 ± 26.1*
Reflex Latency (ms) *from PSTH CUSUM*	All	95.0 ± 12.2	89.1 ± 11.7	96.8 ± 14.8	98.5 ± 13.8
	Active in both conditions	87.9 ± 10.5	87.0 ± 14.3	96.1 ± 14.1	98.7 ± 8.9
Reflex Duration (ms) *from PSTH/PSF CUSUMs*	All	77.8 ± 22.7	89.1 ± 24.6	81.1 ± 27.3	83.2 ± 19.5
	Active in both conditions	81.6 ± 20.0	83.6 ± 25.2	78. ± 26.3	73.8 ± 20.4
Mean ISI (ms)	All	133.9 ± 16.9	130.7 ± 19.1	120.2 ± 15.2	123.4 ± 14.6
	Active in both conditions	128.9 ± 18.9	124.0 ± 16.9	112.0 ± 15.2	114.1 ± 14.1
CV of ISI (%)	All	28.0 ± 9.1	20.6 ± 5.4*	27.1 ± 7.7	21.2 ± 5.4*

Active in both conditions	25.7 ± 7.3	19.4 ± 5.3^{+}	24.3 ± 7.1	20.6 ± 3.9*

* P<0.05; $^{+}$ P < 0.1; comparisons within each MU recording method
HDsEMG – high density surface electromyogram PSTH-peri stimulus time histogram; PSF-peri stimulus frequencygram; CUSUM-cumulative sum; ISI – interspike interval; CV – coefficient of variation.

Table 3. Reflex amplitude and motor unit interspike interval for motor units with comparable firing rate

Parameter	Experiment 1		Experiment 2	
	Free Standing	Lying Supine	Supported Standing	Lying Supine
Number	66	78	14	15
Reflex Amplitude (% max)	-42.0 ± 20.3	-59.0 ± 29.1*	-39.7 ± 23.9	-60.4 ± 31.6*
Mean ISI (ms)	123.8 ± 16.8	125.0 ± 16.3	131.5 ± 13.1	131.3 ± 12.8

Table 4. Ankle and knee joint angles (degrees) during Standing and Lying Supine conditions.

TEST	MEAN		SD	
	Ankle	Knee	Ankle	Knee
Free Standing				
Free Standing	98.1 ± 5.7*	175.2 ± 2.4	0.7 ± 0.5	0.6 ± 0.5
Lying Supine	109.3 ± 4.1	174.4 ± 2.3	0.7 ± 0.5	0.5 ± 0.4
Supported Standing				
Supported Standing	109.8 ± 3.2	174.0 ± 3.8	0.3 ± 0.2	0.3 ± 0.2
Lying Supine	113.4 ± 3.5	172.7 ± 2.6	0.6 ± 0.5	0.2 ± 0.1

*p < 0.05; MEAN-Mean joint angle position; SD-standard deviation of the right leg joint position (describing amplitude of joint movement).

* P<0.05; ISI-interspike interval

Note: Only motor units with inhibition are included.

Figure Captions

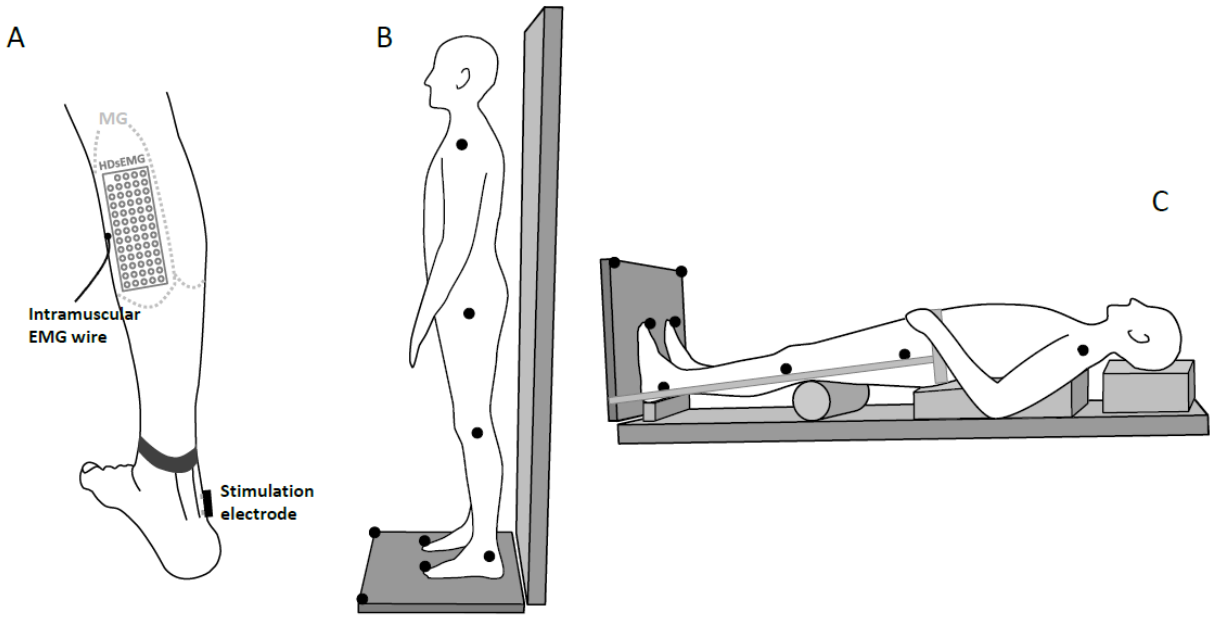

Figure 1. Experimental setup. Positioning of High-Density surface EMG electrode grid and intra-muscular fine wire electrode with respect to medial gastrocnemius muscle (MG; shown with dotted line) on the participant's right leg. The electrode ground strap around the ankle and the bipolar stimulating electrode are shown (A). Position of the tilt table with the mounted force platform for Free Standing (B) and Lying Supine (C) conditions. Reflective markers (black dots) were placed on the force platform and bilaterally on participant's body (13 markers; not all visible). During Lying Supine (C) rigid supports enabled the same relative position of the participant's body and the table as in Free Standing. A waist belt and rigid straps anchored the participant to the force platform. All supports and restraints were removed for Free Standing (B).

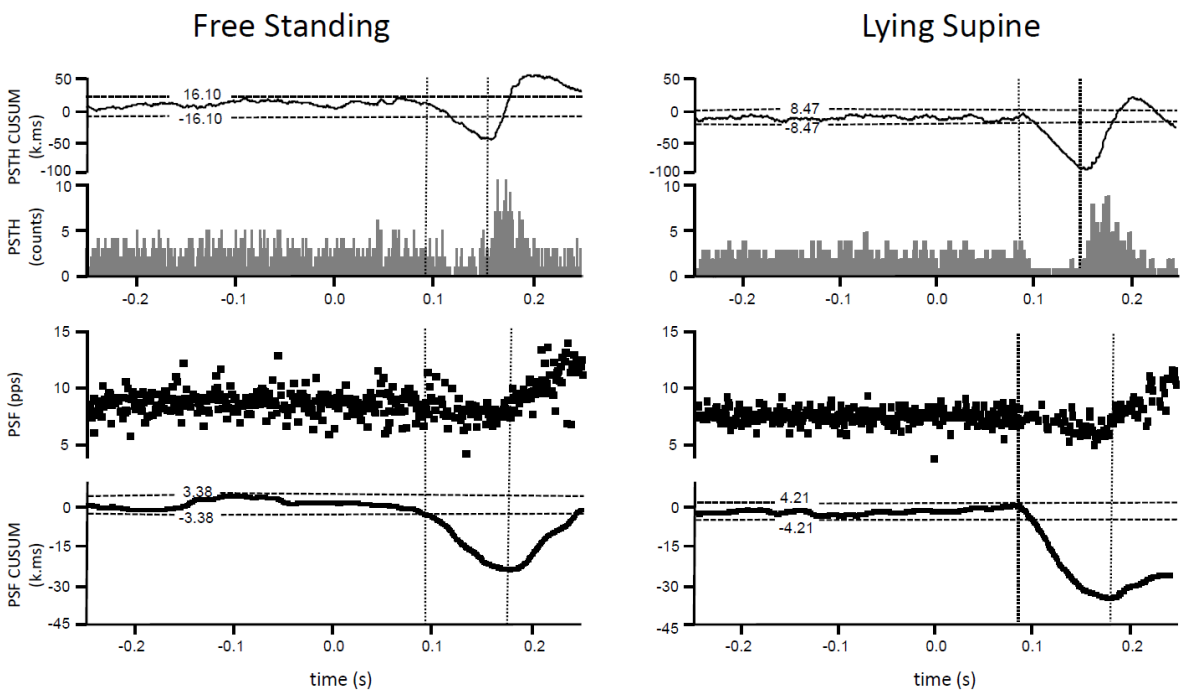

Figure 2. Peri-stimulus time histograms (PSTH) and their cumulative sums (CUSUMs) (top 2 rows) and peri-stimulus frequencygrams (PSF) and corresponding CUSUMs (bottom 2 rows) for the same motor unit decomposed from HDsEMG in Free Standing (left) and Lying Supine (right) conditions. Note the larger inhibition in the Lying Supine Condition.

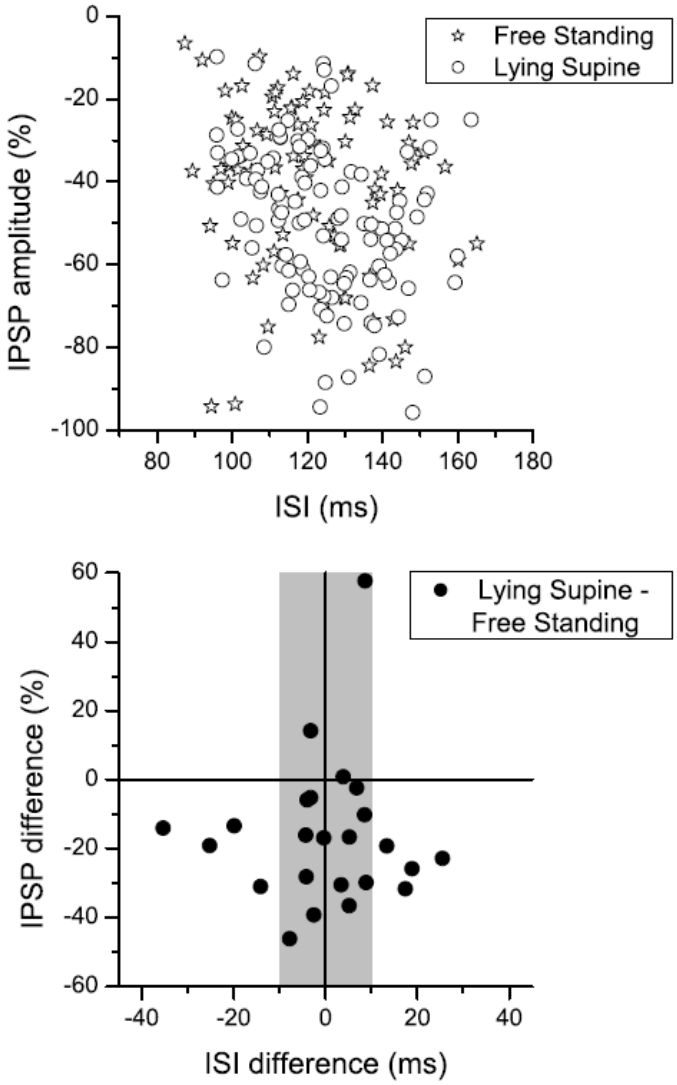

Figure 3. Inhibitory post-synaptic potential (IPSP) amplitude and mean interspike interval (ISI) during Free Standing (☆) and Lying Supine (❍) for all motor units identified with intramuscular and HDsEMG recordings (top panel). There is no relationship between the IPSP amplitude and the ISI (top). For motor units that were active in both conditions, the difference (Lying Supine – Free Standing) of the IPSP amplitude vs. the difference in mean ISI is presented (bottom panel). Motor units with very similar mean ISIs in both conditions (± 10ms) are in the shaded grey area. There is a predominantly negative IPSP amplitude difference for the motor units active in both conditions suggesting larger inhibition during Lying Supine despite the difference in the firing frequency for some MUs.

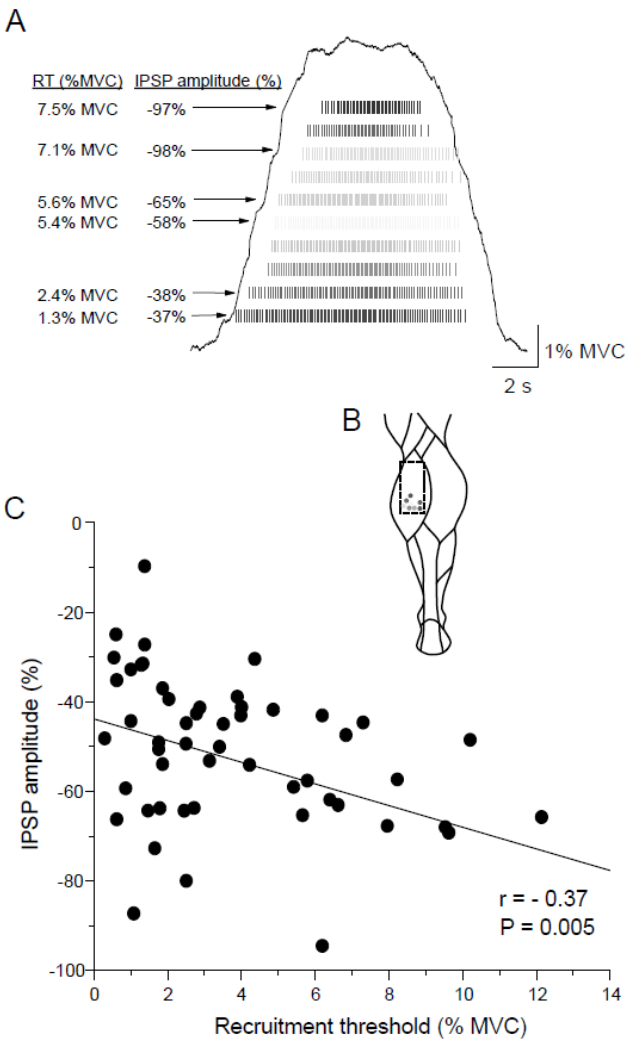

Figure 4. Ramp and hold contraction during the Lying Supine condition from a representative subject (A). The firing times of the single MUs are presented with vertical bars. On the left, the recruitment threshold (RT) and the IPSP amplitude are shown for the motor units (arrows). As it is seen in the top panel, the motor units that were recruited earlier (had lower RT) experienced less inhibition than later recruited motor units. Motor units were recorded from the lower part of the medial gastrocnemius as shown on the lower leg schematic (B). The relationship between the IPSP amplitude and the recruitment threshold for all motor units with the line of best fit is shown in the bottom panel (C). Note: not all MUs that were analyzed for IPSPs were recruited during ramp and hold contractions, hence only 54 out of 103 MUs are plotted.