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Effect of standing posture on inhibitory postsynaptic potentials in gastrocnemius motoneurons

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Running Head: Gastrocnemius motoneuron inhibition in standing

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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

1 **Introduction**

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

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

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

23

24 **Methods**

25 2.1 Participants

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

30 2.2 Electrical stimulation and Electromyography (EMG)

31 Sural nerve stimulation has evoked a robust IPSP in gastrocnemius in past studies (Brooke et al.
32 1997; Khan and Burne 2010; Rogasch et al. 2012). In this study, the sural nerve of the right foot was
33 stimulated through bipolar electrodes (1 cm², 3 cm apart) positioned below the lateral malleolus over
34 the sural nerve trunk. Single square pulse stimuli of 500µs duration were delivered by a constant-
35 current stimulator DS7 (Digitimer Ltd., Hertfordshire, UK) triggered through Power 1401 with Spike 2
36 software (Cambridge Electronic Design Ltd., Cambridge, UK). Perceptual threshold (PT) was determined
37 by increasing the stimulator intensity in 1 mA increments until the participant reported sensation.
38 Stimulator intensity was then reduced until the participant reported no sensation; the last intensity that
39 the participant could perceive was taken as threshold. Reflex stimulation intensity was set at 7 times PT;
40 at this intensity, subjects reported mild pain sensation (3 out of 10).

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

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

52 The HDsEMG grid (semi-disposable adhesive matrix; OTBioelettronica, Torino, Italy) consisted of 64
53 electrodes spaced 8 mm apart, arranged in 5 columns and 13 rows (an electrode missing in one of the
54 corners). Electromyographic signals were collected in monopolar modality using a HDsEMG amplifier
55 (128-channel EMG-USB; OTBioelettronica, Torino, Italy). Signals were amplified 2000 times, sampled at
56 2048 Hz and stored for off-line motor unit action potential extraction. The positions of the fine wire
57 electrode in the right medial gastrocnemius muscle and the HDsEMG grid are depicted in Figure 1A.

58 *2.3 Experimental Protocol*

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

64 Two conditions were tested in each experiment: Free Standing and Lying Supine. The order of
65 the testing conditions (Free Standing or Lying Supine) was randomized. In the Free Standing condition,
66 the tilt table behind the participant was vertical with the foot support parallel to the floor (Figure 1B). In
67 the Lying Supine condition, the tilt table supporting the participant was horizontal and the foot support
68 was vertical (Figure 1C). Before the tilt table was transitioned from Free Standing to Lying Supine,
69 supports were placed between the participant and the table to avoid a change in body position (Figure
70 1C). If Lying Supine was performed first, the straps and supports were removed for Free Standing
71 condition after transitioning slowly from supine to standing.

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

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

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

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

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

105 *2.4. Kinetic and kinematic data.*

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

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

115 *2.4. Data analysis*

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

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

122 For HDsEMG recordings, the single MU action potentials were obtained by decomposition of the
123 EMG signal using DEMUSE software (Holobar and Zazula 2007). Motor unit firing rate (Holobar et al.
124 2010) and reflex inhibition/facilitation (Yavuz et al. 2015) estimated using this method were shown to be
125 valid when compared to gold-standard intramuscular recordings. To identify the MUs that were active
126 in both conditions, epochs from the recordings during each condition were spliced together and
127 decomposed as a single recording and a procedure based on the spatial representation of the motor
128 unit action potential (Dideriksen et al. 2016) was used to verify the correct matching of motor units.
129 Using spike-triggered averaging, the spatial representation of the action potential of each motor unit
130 was obtained for Free Standing and Lying Supine conditions separately. The channels with amplitude
131 higher than 70% of the peak amplitude were identified (Vieira et al. 2010), and the median value of their
132 proximal-distal coordinate was considered to represent the motor unit position. The properties of the
133 action potential identified in both testing conditions were assessed by comparing its spatial
134 representation by calculating the R value of the 2D correlation between average rectified value (ARV)
135 map of the MU action potential in Free Standing vs. Lying Supine condition. As a first step, motor units
136 identified from intramuscular and HDsEMG electrodes were analyzed separately. Motor units were
137 pooled together for further analyses as there was no difference in their firing behaviour between the
138 recording methods.

139 To assess medial gastrocnemius activation, single differential signals were calculated from the
140 monopolar recordings along the columns of the HDsEMG (now 12x5 channels). The differential signals
141 were then filtered with a band-pass filter (Butterworth, 4th order, 10 – 400 Hz) and full-wave rectified.
142 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 (± 250 ms). 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

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

169
$$100\% \text{ reflex amplitude} = (k \times \text{reflex duration in bins}) / \text{number of stimuli},$$

170 where, k is the average prestimulus bin value.

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

174 *2.5. Statistical analysis.*

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

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

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

194

195 **Results**

196 The medial gastrocnemius activation was similar in both conditions (Free Standing: $13.5 \pm 3.5 \mu\text{V}$
197 and Lying Supine: $13.4 \pm 6.3 \mu\text{V}$; N=15; P=0.94 or Supported Standing: $16.1 \pm 11.0 \mu\text{V}$ and Lying Supine:
198 $15.1 \pm 7.4 \mu\text{V}$; N=4; P=0.63) The stimulus intensity (Free Standing: $40.2 \pm 6.4 \text{ mA}$ and Lying Supine: 40.5
199 $\pm 7.4 \text{ mA}$; N=15; P=0.70 or Supported Standing: $45.7 \pm 8.9 \text{ mA}$ and Lying Supine: $45.6 \pm 8.8 \text{ mA}$; N=4;
200 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
201 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
202 conditions.

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

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

215 the MU action potential between Free Standing and Lying Supine (0.94; 25th – 75th percentiles: 0.89-
216 0.96), confirming that the same motor unit was being recorded in both conditions.

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

229 To explore possible explanations for this finding, we sought to determine if differences in motor
230 unit firing rate between the two conditions might influence the results. There was no significant
231 relationship between the strength of the inhibition and the mean ISI for all motor units, during both,
232 Free Standing and Lying Supine, conditions ($r = -0.09$; $P = 0.3$ and $r = -0.06$; $P = 0.5$, respectively; Figure 3
233 top). To eliminate the possibility that small differences in firing rate could affect the strength of the
234 inhibition, for motor units that were active in both conditions we plotted the difference in IPSP
235 amplitude against the mean ISI difference between Lying Supine and Free Standing conditions (Figure 3
236 bottom). Figure 3 shows that the strength of inhibition was unaffected by ISI ($r = 0.03$, $P = 0.8$).
237 Whether the mean ISI was within 10ms between conditions (shaded region Fig 3 bottom), higher or
238 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 ($19.5 \pm 4.8\%$ and $17.8 \pm 2.9\%$ 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 ($-58.6 \pm 30.3\%$ and $-39.7 \pm 20.7\%$ 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, 25th – 75th 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 ($r = -$

262 0.37; P=0.005) between the strength of the inhibition (negative number to indicate inhibition) and the
263 recruitment threshold (Figure 4 C).

264

265 **Discussion**

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

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

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

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

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

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

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

315 We tried to keep the overall level of central drive similar between conditions by asking
316 participants to control the discharge rate of the intramuscularly-recorded motor unit between 6-8 Hz (ISI
317 125-166ms). On average, the motor unit ISI recorded from the HDsEMG grid was slightly faster than 8
318 Hz – an ISI of around 120ms in both Free Standing and Lying Supine for all units. Overall the motor units
319 recorded during Lying Supine were not significantly slower than the Supported Standing condition and
320 Figure 3 shows no relationship between firing rate and IPSP strength from both intramuscular and
321 HDsEMG recordings. This, along with the additional analysis in Table 4, suggests that IPSP strength is
322 not solely a function of motor unit discharge rate.

323 In human experiments, we only have a proxy of central drive in the recordings of net motor unit
324 discharge rate. In mammalian preparations, Berg et al. (2007) showed a balance of excitatory and
325 inhibitory inputs in spinal motoneurons; that is, as excitation was increased, so was inhibition.
326 Therefore it is possible that similar motor unit firing rates in both Standing and Supine conditions are
327 due to increased excitation in standing accompanied by increased inhibition. In cortical neurons,
328 neurons experiencing greater synaptic activation were in a higher conductance state (Bernander et al.
329 1991; Destexhe et al. 2003). This high conductance state could result in shunting of additional inhibitory
330 currents presented by the sural nerve stimulation in the Standing conditions that was not seen in Lying
331 Supine.

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

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

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

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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*
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* 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

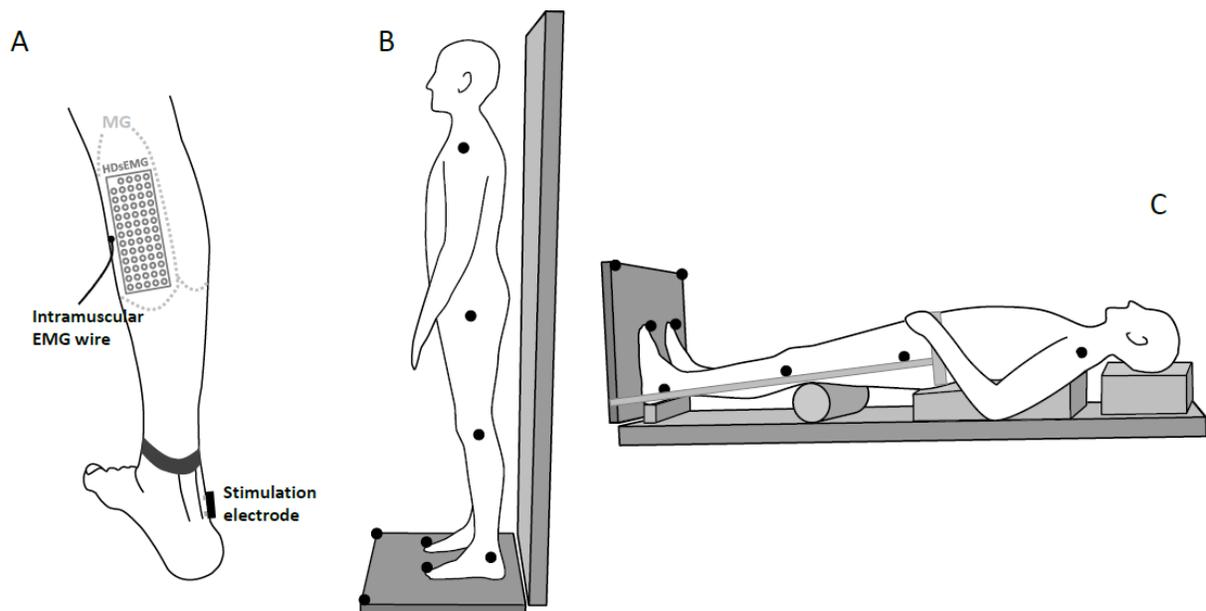


Figure 1. Experimental setup. Positioning of High-Density surface EMG electrode grid and intra-muscular fine wire electrode with respect to medial gastrocnemius muscle (MG; shown with dotted line) on the participant's right leg. The electrode ground strap around the ankle and the bipolar stimulating 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).

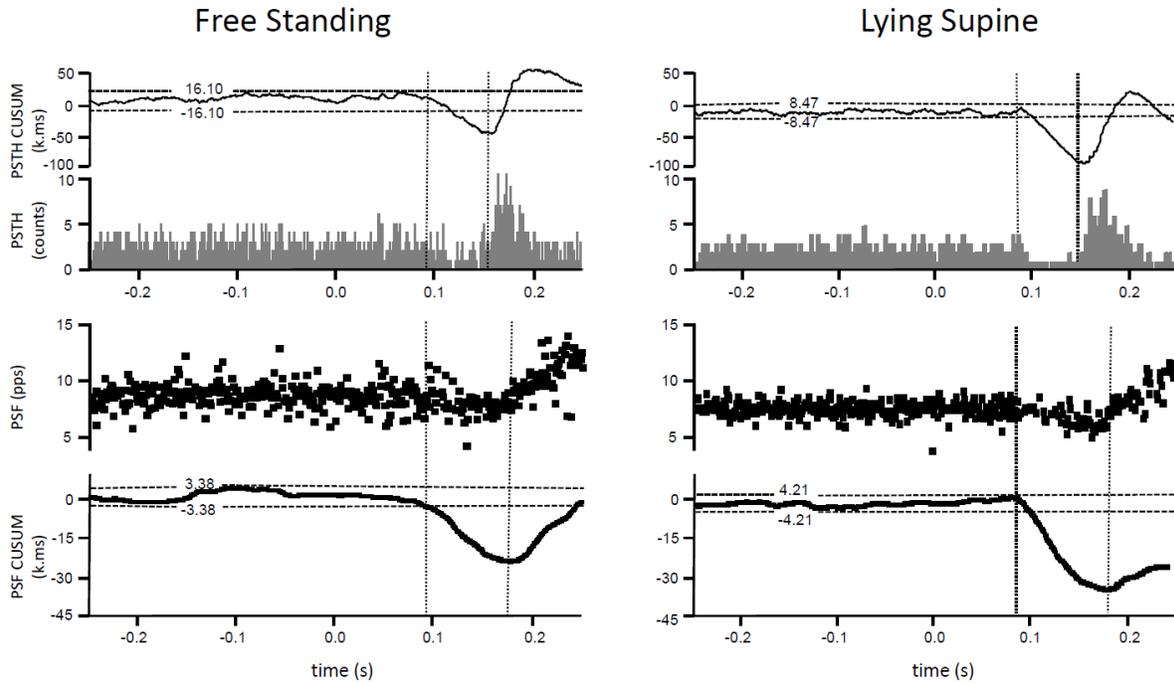


Figure 2. Peri-stimulus time histograms (PSTH) and their cumulative sums (CUSUMs) (top 2 rows) and peri-stimulus frequencygrams (PSF) and corresponding CUSUMs (bottom 2 rows) for the same motor unit decomposed from HDsEMG in Free Standing (left) and Lying Supine (right) conditions. Note the larger inhibition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

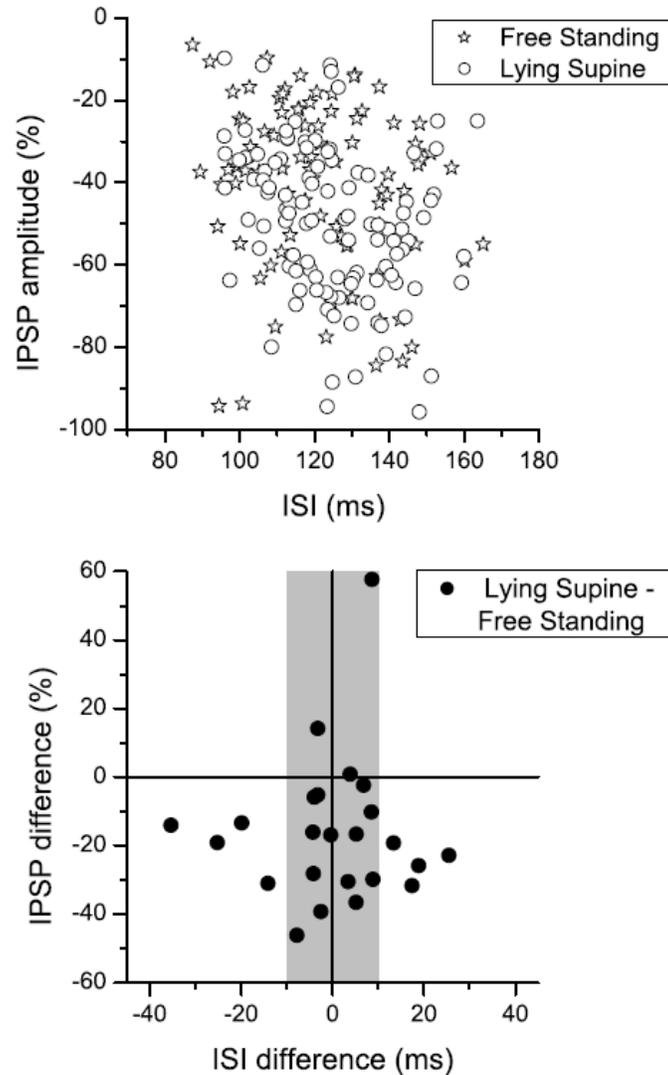


Figure 3. Inhibitory post-synaptic potential (IPSP) amplitude and mean interspike interval (ISI) during Free Standing (☆) and Lying Supine (○) for all motor units identified with intramuscular and HDsEMG recordings (top panel). There is no relationship between the IPSP amplitude and the ISI (top). For 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 (± 10 ms) 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.

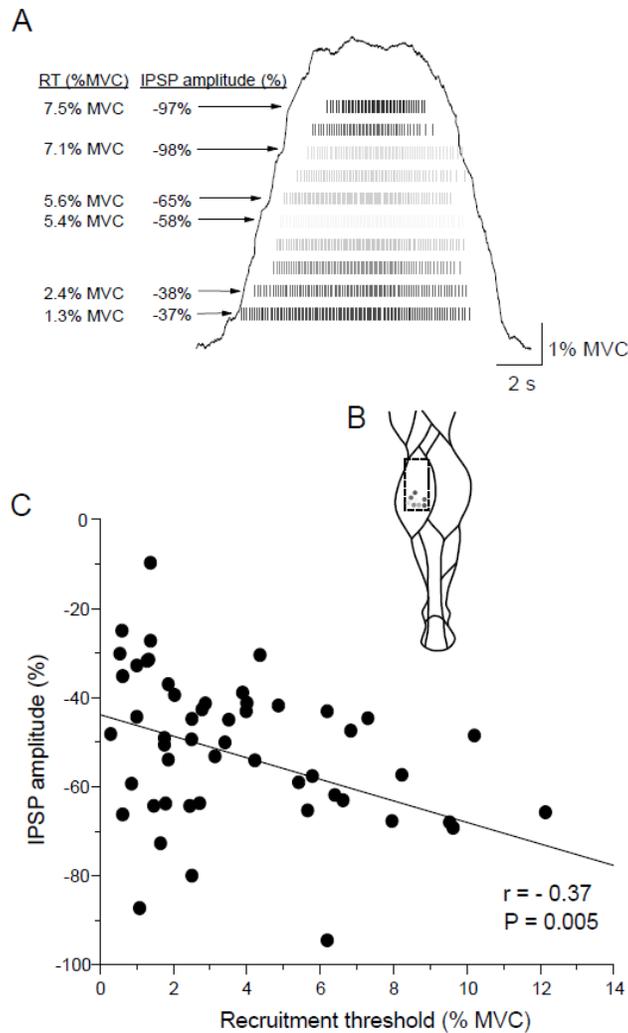


Figure 4. Ramp and hold contraction during the Lying Supine condition from a representative subject (A). The firing times of the single MUs are presented with vertical bars. On the left, the recruitment threshold (RT) and the IPSP amplitude are shown for the motor units (arrows). As it is seen in the 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.

