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Title: Recruitment of motor units in the medial gastrocnemius muscle during human quiet standing: Is recruitment intermittent? What triggers recruitment?

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Abstract

Compensation of impending micro-falls when humans stand at ease has been shown to depend on the sluggish triggering of impulsive shortenings of the medial gastrocnemius (MG) and soleus muscles. Here we test whether it is mainly the recruitment or the modulation of discharge rate of motor units to provide the impulsive regulation of postural ankle torque. The number of active motor units, rather than their rate coding, is expected to meet the requirement of adjusting ankle torque impulsively. While seven healthy subjects stood quietly, intramuscular electromyograms were recorded from the MG muscle with three pairs of wire electrodes. The number of active motor units and their mean discharge rate were compared for different sway velocities and positions. Motor unit discharges occurred more frequently when the body swayed faster and forward (Pearson $R = 0.63$; $P < 0.0001$). This higher likelihood of observing motor unit potentials was explained chiefly by the recruitment of additional units. From slow, backward sways to fast, forward body shifts, the mean number of units active increased from one to nine (MANOVA, $P < 0.0001$) whereas the discharge rate changed from 6 ± 1.2 ($\pm$s.d.) to 10 ± 0.9 pps (MANOVA, $P = 0.001$). Strikingly, motor units did not discharge continuously throughout standing. They were recruited on the initiation of forward sways and intermittently, with a modal frequency of two recruitments per second. This modal interval between recruitments (~500 ms) is associated neither with the modal duration of postural sways (~2s) nor with the delay of reflexive control (~100 ms). The modal rate of two recruitments per second is more likely related to the psychological refractory period, which is associated with higher level limitations in the planning and control of actions.

Introduction

When standing upright quietly, the human body sways incessantly. These tiny and spontaneous movements are not easily observed by the naked eye and barely exceed the visual and proprioceptive thresholds of consciousness (Fitzpatrick & McCloskey, 1994). Because the standing body is unstable, any small shift of the centre of gravity (CoG) from the ankle axis of rotation threatens the body stability, with larger shifts impinging stronger destabilising moments. Because in natural standing posture the position of body CoG is forward of the ankle joint (~5cm, Hellebrandt *et al.* 1938), relentless actions are sought to avoid falling forward. The inherent elasticity of the Achilles tendons and of the calf muscles is not sufficient to compensate fully for the gravitational toppling torque (Loram & Lakie, 2002a; Casadio *et al.* 2005).

Regulation of standing is, indeed, associated with active adjustments in the ankle torque. Contrary to the conservative reasoning that the forward, postural sways stretch the ankle extensors (Hellebrandt, 1938), variations in the length of the calf muscles and in body position are not orthodox. The medial gastrocnemius (MG) and the soleus muscles shorten while the body sways forward (Loram *et al.* 2005). Calf muscle shortenings appear to result from a series of ballistic actions, executed sluggishly ~ 400 ms (Loram & Lakie 2002b; Loram *et al.* 2005). How the activation of the calf muscles is triggered is uncertain.

Active gradation of muscle force depends on the temporal (rate coding) and spatial (recruitment) summation of motor unit action potentials. Whether one of these mechanisms predominates over the other depends on the situation. It is well established, for example, that small motor units, with a few muscle fibres, are the first to discharge when the muscle is stretched (Henneman, 1965). With the progression of stretching, the active units increase their rate of discharge and additional, larger units are recruited eventually. This same sequence of events has been observed for voluntary isometric contractions (Bigland & Lippold, 1954; DeLuca *et al.* 1982; Person & Kudina, 1971). Nevertheless, as the muscle architecture and the mode of contractions might both shape the recruitment of motor units (Howell *et al.* 1995; Kato *et al.* 1985; Kennedy &

Cresswell, 2001; Tax *et al.* 1989; Vieira *et al.* 2011), it is unclear whether rate coding or recruitment contributes more to the regulation of gastrocnemius force during standing.

In this study we ask whether the recruitment or the discharge rate of motor units in the human MG muscle is the predominant mechanism for the control of ankle torque during standing. Three possibilities could account for the generation of impulsive ankle torque in standing: i) Short periods of higher discharge rate of the MG motor units, interposed between periods of lower rate; ii) Besides, or in addition to, the variation in the discharge rates of active units, additional motor units of higher threshold are re- and de-recruited in short bursts; iii) Motor units are switched *on* and *off*, intermittently throughout standing. The third condition imposes periods when MG motor units are *off* and the muscle is then silent (Asai *et al.* 2009), whereas the two former conditions imply continuous MG muscle activity throughout standing (Maurer & Peterka, 2005; van der Kooij & de Vlugt, 2007). Given the phasic nature of the gastrocnemius muscle, especially in relation to the tonic soleus muscle (Di Giulio *et al.* 2009; Joseph *et al.* 1955; Mori, 1973), the intermittent recruitment of motor units is expected to account for the postural activation of the MG muscle.

If gastrocnemius force in standing is regulated by recruitment then why or when are the motor units recruited? What mechanism triggers these units? Does the triggering depend on the body state (i.e., position/velocity)? Does it depend on a temporal process in the nervous system that is unrelated to motion of the body (i.e., some sort of internal irregular clock or cycle)? Previous, experimental evidence shows that: i) during standing, 2-3 muscle impulses are produced per second (Loram *et al.* 2005); ii) during visual manual control of an inverted pendulum subjects adopt a modal rate of 2-3 actions per second when controlling position and velocity (Loram *et al.* 2011); iii) when the load time constant is altered, there are still 2-3 control movements per second (Loram *et al.* 2006). This latter observation supports the notion that the modal rate of actions is regulated according to an intrinsic temporal process rather than motion of the body, which would depend on the time constant of the load. Therefore, we further ask whether there is evidence of recruitment with a modal rate of two-three recruitments per second and/or whether recruitment is related to motion of the load/body.

The mechanism governing the recruitment of MG motor units in standing, together with the main issue on the control of motor units, are summarised in three main questions addressed in this study: i) Does the number of action potentials of individual motor units increase with sway position and/or velocity? ii) What is the cause of variations in the number of discharges; is it the recruitment of additional units, the increase of the discharge rate of active motor units, or both? iii) If the recruitment of motor units accounts for the variation of standing ankle torque, then, what accounts for when the motor units are recruited?

Methods

Subjects and protocol

Nine healthy subjects (24 – 35 years; 152 – 181 cm; 56 – 82 kg; one female) volunteered to stand at ease for 60 s, with their arms resting alongside the body and their feet in parallel and comfortable position. All subjects provided written informed consent before participating in the study. The experimental protocol conformed to the latest amend of the *Declaration of Helsinki* and was approved by the Ethical Committee of the Region North Jutland, Denmark.

Eyes open and eyes closed trials were applied twice, with 2 min interval. As the body sways change with intention (Loram & Lakie, 2002b), subjects were instructed to not concentrate on the task. To ensure that subjects were distracted from the standing task, they were engaged into active conversation, without changing their body orientation. As the visual input did not affect the recruitment and discharge rate of motor units, the eyes open and the eyes closed data were collapsed.

Experimental setup

Detailed information on the recording of EMGs was given in a previous paper (Vieira *et al.* 2011). In brief, intramuscular EMGs were recorded with three pairs of Teflon-coated stainless-steel wire electrodes (0.2 mm diameter; A-M Systems, Carlsborg, WA), inserted into three locations along the MG muscle with a 25-gauge hypodermic needle. Given the pinnate geometry of the muscle, each pair of electrodes was inserted into a different group of muscle fascicles (Figure 1). Firstly, the location at which the most distal fascicle in the MG muscle attaches to the superficial aponeurosis was identified with ultrasound imaging and marked on the skin (Figure 1). After that, the distance d between this location and the popliteal fossa was measured. Finally, to account for differences in the leg length between subjects, the pairs of electrodes were separated from each other by $0.15d$, with the first pair being inserted $0.1d$ above the distal extremity of the superficial aponeurosis (Figure 1; dashed line). The locations of EMG recordings were classified as $MG_{90\%}$ ($0.1d$ above the distal extremity of the superficial aponeurosis), $MG_{75\%}$ ($0.15d$ proximal to $MG_{90\%}$ location) and $MG_{60\%}$ ($0.3d$ proximal to $MG_{90\%}$ location).

Amplification of intramuscular EMGs varied between 500 and 5000, to provide the highest signal-to-noise ratio without saturation of the action potentials (10 Hz – 5 kHz bandwidth; EMG-USB2, OT Bioelettronica, Italy). Horizontal and vertical ground reaction forces were measured by using a force-plate with four strain-gauge sensors (OR6-7 AMTI, Watertown, MA). Forces and EMG signals were synchronously acquired with a 12 bit A/D converter (± 2.5 V dynamic range) and sampled at 10 kHz. The position of the body centre of pressure (CoP) was calculated from the ground reaction forces.

Decomposition of EMG and estimation of body position and velocity
Initially, intramuscular EMGs were filtered between 0.1 and 1.5 kHz with a digital Butterworth filter (2nd order; zero-phase distortion). Individual trains of motor unit action potentials were identified by first automatically decomposing the filtered signals with the tool EMGLAB (McGill *et al.* 2005). Then, missed potentials, or potentials obscured by the superimposition of the activity of different motor units, were corrected manually. The firing pattern information concerning the EMGs recorded from the three locations along the MG muscle were pooled to investigate how the motor units are activated for the stabilization of body sways in quiet standing.

Modulations in recruitment and discharge rate of motor units during quiet standing were evaluated in the phase plane (velocity versus position of the body). After decimation of the CoP time series to 20 Hz, the position of the body centre of gravity (CoG) was estimated with the method of low-pass filtering (Caron *et al.* 1997). CoG velocity was computed by differentiating CoG position. Albeit the method of low-pass filtering performs marginally worse for the estimation of CoG position, when compared with the technique of double-integration of shear forces (Lafond *et al.* 2004), low-pass filtering does not constrain the CoP and CoG positions to coincide for instants of zero horizontal acceleration. Such a constraint produces discontinuities in the CoG position (unpublished observations).

Motor unit activity in the phase plane

The contribution of motor units in the MG muscle to the control of body sways was investigated, initially, by quantifying how frequently a motor unit discharged at a certain body position and velocity. The distribution of CoG position and velocity was partitioned into ten bins, each comprising 10% of the samples. The first and the last bins were excluded to avoid the counting of discharges for uncommon body states. Instants of motor unit discharge were used to interpolate the time series of CoG position and velocity and, thus, to identify to which bin each discharge corresponded.

Predominance of either recruitment or discharge rate in body stabilisation during quiet standing was assessed in the phase plane. The number of active motor units and their discharge rate were quantified for the bi-dimensional distribution of CoG position and velocity. A motor unit was deemed to be active for a specific body state if one discharge, at least, occurred within one of the eight percentiles of the distribution of CoG position and velocity. The discharge rate of active motor units was calculated as the reciprocal of inter-pulse intervals (i.e., period between successive discharges). For each subject and for each of the 64 bins in the phase plane, the number of active motor units and their discharge rate were averaged. In the case none motor units discharged within a specific bin, the number of active units was considered to be zero and the mean discharge rate was not computed.

Statistical analysis

Variations in the mean discharge rate and in the number of active motor units during standing were tested using a multivariate analysis of variance MANOVA (8 position bins x 8 velocity bins). Parametric testing was applied after we ensured the variables to distribute normally across CoG position and velocity. Once the statistical significance was verified, the degree of dependence of the number of active motor units and of their discharge rate on the sway position and velocity was tested with the Spearman (for the number of units) and Pearson (for the discharge rate) correlation. For clarity, the statistics used to compare the features of intermittent activity of motor units are stated in the Results section. Group data are presented with mean and standard deviation.

Results

Summary of results

On average, twelve motor units per subject were identified in the intramuscular EMGs recorded from the three locations in the MG muscle while subjects stood at ease. None of the active units discharged continuously during the whole standing duration. The likelihood of observing motor unit action potentials increased linearly with the forward sway velocity (Pearson $R = 0.40$; $P = 0.002$) and with the forward body position ($R = 0.63$; $P < 0.0001$). This increase was explained, mainly, by the recruitment of additional motor units when the body moved forward or when it moved faster (MANOVA, $P < 0.0001$ in both cases; N = 8 bins of position and velocity). The discharge rate of the active motor units did not depend on the body position (MANOVA, $P = 0.47$; N = 8 bins) and showed a somewhat abrupt increase with sway velocity (MANOVA, $P = 0.02$; N = 8 bins). Strikingly, motor units were recruited intermittently during standing, with a modal frequency of two recruitments per second. These results are detailed in the following.

Periodical activity of motor units during standing

Intramuscular EMGs were recorded from the MG muscle to investigate whether modulations in the activity of motor units accounts for the active control of human standing posture. If the activation of MG motor units does not relate to the control of bodily sways, then, the number of motor unit action potentials would not change throughout standing.

Results of a representative subject, shown in Figure 2, reveal that MG motor units did not discharge continuously in the standing position. Periods of motor units silencing ($\geq$ 1 s) were alternated with similar periods of activity (see EMGs and stabilometric data in Figure 2*A*). The alternation between silencing and firing was observed for all the eight motor units identified from the three EMGs in this subject (see firing patterns in Figure 2*B*). Close inspection of Figure 2 suggests the discharges of motor units to occur more frequently when the body position locates more forward (i.e., the spikes in the EMGs and the firing pattern of individual units match the instants in which the body CoG is forward).

In fact, motor units discharges distributed unevenly in the phase plane representation of the bodily sways. Figure 3 shows the phase plane representation for two representative subjects. Each coordinate in the phase plane (sway velocity × sway position) corresponds to a single motor unit discharge. The average velocity and the average position of the body during the whole standing test correspond to the origin in the phase plane. Activation of motor units in the MG muscle followed consistent trajectories in the phase plane, residing chiefly in the first and fourth quadrants. These quadrants define regions of transition in the direction of CoG sways, from the forward to the backward direction. Dense clusters of dots in Figure 3 are more frequent as the position of the body CoG is progressively more forward. Although such an increase in the concentration of dots with the sway velocity is less evident, it indicates some reliance of motor units activity on the body position and velocity during standing.

Motor units discharges are regulated by the sway velocity and position
Averaged data across all participants indicated that the likelihood of an individual motor unit to discharge was significantly related to the sway velocity and position (ANOVA, $P = 0.0001$; Figure 4). Subjects from whom more motor units were identified during standing were more likely to show a high number of discharges across different body states. For this reason, it was necessary to normalise the total number of discharges occurring within each bin of velocity and position by the number of motor units identified (Figure 4). Indeed, individual motor units discharged progressively more as the body moved faster in the forward direction (Pearson $R = 0.40$; $P = 0.002$) and when the body position shifted from back to forward ($R = 0.63$; $P < 0.0001$; dashed lines in Figure 4). The increase in the number of discharges with sway velocity was less steep when compared with sway position, as motor unit discharged less frequently for the extremely fast body movements in the forward direction (see the two greatest percentiles in Figure 4).

Recruitment and rate coding: which predominates during standing?
The recruitment of additional motor units, the increase in the firing rate of active units, or both mechanisms, might explain the greater number of discharges with the forward body shifts and with the faster forward movements (Figure 4). We are interested in

knowing whether one controlling mechanism predominates over the other. If the regulation of body sways results chiefly from the recruitment of motor units, then, the number of active units, but not their rate of discharge, would vary with the sway velocity and position. It is worth noting here that the selective wires that we used for EMG recording obviously limit the detected motor units to a smaller number than the active units. When we refer to number of recruited motor units in the following, we thus mean the number of motor units recruited that could be detected by the recording wires. This limitation of the approach, which is common to all motor unit studies, does not influence our conclusions (see Discussion).

The average number of motor units active and their average discharge rate distributed unevenly in the phase plane (Figure 5). Contour lines in Figure 5 denote regions in the phase plane where the number of active units or their discharge rate changes (Figure 5*A,B*). The recruitment of motor units varied significantly with either body position or velocity (MANOVA, $P < 0.0001$ in both cases; N = 8 bins). From the end of a backward sway (third quadrant in the phase plane) to the end of forward body movement (first quadrant), the mean number of active motor units increased from one to nine (Figure 5*A*). Assuming a similar discharge rate at recruitment for all motor units, this rate of recruitment implies an increase by 900% in number of discharged action potentials. Considering this same trajectory in the phase plane, the average discharge rate of active units accounted for only a 67% increase in number of action potentials (from 6 pps to 10 pps; Figure 5*B*). This variation in rate coding was significant across sway positions (MANOVA, $P = 0.05$; N = 8 bins) or sway velocities (MANOVA, $P = 0.001$; N = 8 bins). Interestingly, motor units discharged more frequently in the second and first quadrants, with a somewhat decrease in their discharge rate in-between these two regions (Figure 5*B*). Interaction between position and velocity bins did not influence the recruitment nor the rate coding of MG motor units (MANOVA, $P > 0.5$ in both cases; N = 64 bins).

Although both mechanisms were significantly related to the position and velocity of the body, the recruitment of motor units rather than rate modulation was more strongly associated with the control of bodily oscillations. The mean number of motor units

active correlated more strongly with sway velocity and position (Figure 6*A)* when compared to the average discharge rate (Figure 6*B*). With the increase in sway velocity or position, the number of active units trebled. The relative increase in the discharge rate of active units with the sway velocity or position was not higher than 50% (compare the average discharge rate between the third and eighth percentiles in Figure 6*B*). This relatively small increase in discharge rate was observed even when excluding the motor units recruited for velocity and position percentiles greater than 30% from the computation. Actually, individual motor units rarely discharged at rates higher than 12 pps (Figure 7*A*).

Intermittent activation of motor units and its representation in the phase plane
Given the predominance of recruitment of MG motor units in standing, we further ask which mechanisms might have triggered the recruitment of these units. One possibility is that recruitment occurs at a regular interval (i.e., internal mechanism of triggering). Alternatively, recruitment occurs whenever a state dependent threshold has been passed (i.e., event related mechanism). Our results indicate that recruitment depends on both mechanisms, as detailed in the following.

Very frequently, motor units discharged below the physiological, minimal tonic rates (< 4 pps; Figure 7*A*). These occurrences were considered as representing derecruitment followed by re-recruitment of motor units. Despite the high number of occurrences for instantaneous discharge rates ranging from 7 to 11 pps, about 20% of the values for instantaneous discharge rate were distributed between 1 and 4 pps (total number of cases equals 7164; Figure 7*A*). The interval between successive motor unit recruitments did not distribute uniformly during standing. Strikingly, the histogram shown in Figure 7*B* indicates a clear, modal interval between successive motor unit recruitments (500 ms; i.e. individual motor units were preferentially recruited twice every second).

The instants of motor unit recruitment had, in addition, a definite representation in the phase plane, with motor units being triggered chiefly in the first quadrant (Figure 7*C*). On average, events of motor unit recruitment were more likely to occur as body position shifted forward ($P = 0.0001$, $N = 7$ subjects). Although less strongly, the body velocity

also affected the probability of triggering motor units (P = 0.02, N = 7 subjects). Interestingly, motor units were very rarely recruited in the third quadrant (i.e., when subjects swayed backward; Figure 7*C*).

Discussion

When subjects stand quietly, their spontaneous bodily sways are regulated with a series of impulsive adjustments in ankle torque (Loram *et al.* 2005). The recruitment of motor units, the temporary modulations in the rate coding of motor units, or both, might account for the production of impulsive, postural ankle torque. From intramuscular EMGs of seven subjects, we observed that stabilisation of standing posture relies mainly not on the discharge rate but on the number of units active in the MG muscle (Figure 5 and 6). Also, motor units were recruited at specific regions in the phase plane and with a regular interval (i.e., two recruitments per second; Figure 7). These results indicate that postural activation of the MG motor units is not continuous. It consists in a series of discrete, sluggish and regular actions triggered according to the position and velocity of the standing body.

Recruitment or discharge rate: which compensates for the body sways?
Our results indicate that ankle torque in quiet standing is controlled by the selective mobilisation of varying number of small units. When the body swayed in the backward direction, motor units in the MG muscle were switched off progressively. The number of motor units active decreased from the fourth to the third quadrant in the phase plane (see Figure 5*A*). Once the subjects initiated a forward sway (i.e., gravity accelerates the body forward), falling was prevented not with the rate coding of the active units but with the recruitment of motor units. The more forward the body swayed, the higher the number of units activated (see Figure 5*A*, from the second to the first quadrant). Active contribution of the MG muscle to the ankle torque in standing is preferentially due to the recruitment of motor units.

The predominance of recruitment over rate coding during standing is likely accounted for by stabilisation demands. When subjects are asked to contract their muscle isometrically, they show a preferential reliance on the discharge rate for the regulation

of muscle force, with the firstly recruited (smallest) units typically reaching higher peaks of instantaneous firing rate (Bigland & Lippold, 1954; DeLuca *et al.* 1982; Person & Kudina, 1971). In virtue of their low recruitment threshold, the postural motor units in the MG muscle are likely able to discharge at low as well as at high rates. Then, one would expect the rate coding of MG units to have a more significant contribution, than it apparently has, to the compensation of bodily sways (Figure 5 and 6). However, MG contraction in standing is nearly isometric, with tiny variations in muscle length occurring in standing (Loram *et al.* 2005). Also, the bipedal, standing body provides the nervous system with a short interval for the issuing of stabilising actions (i.e., the time constant of the falling body ranges from 0.50 to 0.92s, depending on the passive muscle-tendon stiffness which is limited by the Achilles tendon stiffness; Jacono *et al.* 2004; Loram *et al.* 2006, 2009). For this reason, compensation of the forward, bodily sways demands prompt rather than smooth increases in the torque of ankle extension. While the rate coding would take time to grade ankle torque, recruitment is intrinsically impulsive (Mariani *et al.* 1980); it allows for the instantaneous gradation of stabilising ankle torque in standing (see variations in CoP position with respect to the firing pattern of motor units in Figure 2). The discharges of a population of briskly recruited units 'abruptly throw the muscle into action' (Desmedt & Godaux, 1977; p. 689), likely benefiting from the intensification of the muscle *active state* (i.e., greater capacity of the muscle to develop tension) for the sudden regulation of muscle force (Desmedt & Hainaut 1968). Indeed, the lowering of the recruitment threshold of motor units, observed in dynamic contractions, is an evidence of the importance of recruitment at an early stage of the movement (Theeuwen *et al.* 1994; Tax *et al.* 1989), in particular when the movement is performed ballisticaly (Desmedt & Godaux, 1977). Therefore, recruitment of motor units is better suited than rate coding for the stabilisation of spontaneous sways occurring in human quiet standing.

Muscle anatomy might also explain the preference for the recruitment of motor units in regulating the ankle torque during standing. When subjects are asked to vary the muscle force linearly to some percentage of their maximum, for example, the relative contribution of recruitment and rate coding appears to depend on the muscle anatomical size. DeLuca *et al.* (1982) observed that while motor units in the small first dorsal

interosseous muscle increase their discharge rate smoothly to regulate the force of finger abduction, the linear increase in force produced by the larger, deltoid muscle shows greater reliance on the recruitment rather than on the rate coding. Consequently, the more motor units a muscle has, the finer the recruitment of units seems to account for the gradation of its force. The gastrocnemius is a large muscle, containing a larger population of muscle units (~550 units) when compared to other small muscles, like the first dorsal interosseous (~100 units; Feinstein *et al.* 1955). In virtue of this considerably large number of motor units in the MG muscle, the individual contribution of each unit to the muscle force posits a small fraction of the muscle maximal force. Interestingly, the motor units in the MG muscle of the cat show a broad spectrum of tetanic tension, ranging from 0.5 g to 120.0 g (Wuerker *et al.* 1965). Additionally, these authors observed that the most excitable units in the cat MG produced smaller values of mean tetanic tension (4.9 g) than those residing in the homogeneous soleus muscle of the cat (6.8 g). Therefore, the recruitment of motor units in the MG muscle during standing suits not only for the immediate, but also for the small regulation of ankle torque.

Notwithstanding the fact that control of MG force is predominantly due to the recruitment of motor units, small variations in rate coding occurred while subjects stood at ease (Fig. 6). Is this slight, significant change in rate coding functionally relevant? Considering that motor units were active during brief intervals (median duration: 0.49 s; interquartile interval: 0.20 – 1.31 s; N = 1292 bursts), the average increase of two pulses per second (Fig. 5 and 6) would lead to one additional discharge per unit. Conversely, the recruitment of one additional unit would result in four additional discharges; the number of additional discharges per unit is the product between its average discharge rate and duration (i.e., 8 pps multiplied by 0.49 s yields about four discharges per unit). Regardless of whether the variation in rate coding occurred within or between the short intervals of MG activity, its contribution to the total ankle torque in standing is functionally marginal with respect to that resulting from the recruitment of motor units. Additionally, it is worth mentioning that we analysed a subsample of the active, postural units. Because we used selective wire electrodes, the number of detected units is much less than the number of active units. Having looked at subsample of active units, however, does not influence the conclusion of predominance of recruitment over

discharge rate for the regulation of MG force in standing. Indeed, it indicates that the number of recruited units is much larger than we can detect so that the predominance of recruitment over modulation in discharge rate would be even more evident if a greater sample of motor units could be detected.

When recruitment occurs? What theories of control of standing is it relevant to?
Understanding how the nervous system stabilises the human quiet standing posture has been the subject of several theoretical investigations. It is commonly accepted that sensing the body state and issuing appropriate signals, after some delay, to the ankle extensors are both mechanisms involved in stabilisation. On the other hand, the nature of these signals and of how these signals are triggered is controversial. One possibility posits the continuous regulation of ankle torque during standing (i.e., the calf muscles are active uninterruptedly; Peterka, 2002; Maurer & Peterka, 2005; Masani *et al.* 2006; van der Kooij H & de Vlugt E, 2007). Alternatively, some authors suggest the control of standing posture to depend on intermittent torque pulses, triggered either according to specific body position and velocity states (Asai *et al.* 2009; Bottaro *et al.* 2008) or to some intrinsic, internal temporal process that takes a characteristic time (e.g., a modal time of ~ 0.5 s, (Loram *et al.* 2011)). Our results show that activation of the MG muscle in standing is intermittent and not continuous, with the recruitment of motor units depending both on the body position and velocity state and on an internal process (Gawthrop *et al.* 2011).

Intermittency of MG activity during standing manifested in two forms, with intermittent recruitment of units occurring at long (>1s) and short (~500 ms) intervals (see Figures 2 and 7). Interestingly, periods of active silencing longer than 1s coincided consistently with the periods of backward sways (Figure 7*C*). The fact that, in quiet standing, the body centre of gravity is ahead of the ankle joint (Hellebrandt, 1938), in addition to the absent recruitment of motor units in the MG muscle at specific body states (Figure 7*C*), are evidences supporting the deactivation of ankle extensors when the body sways backward. In the more backward position, activation of ankle extensors would threat stability by eventually slanting the body further backward over the ankle. Reversion of backward sways could in principle be due to active intervention of ankle flexors (e.g.,

tibial anterior muscle) or due to gravity. The former possibility is unlikely to occur, as EMGs recorded from ankle flexors in standing rarely show potentials (Di Giulio *et al.* 2009; Joseph *et al.* 1955). The nervous system, then, appears to take advantage of the pull of gravity to provide an economical solution for the stabilisation of the unstable, standing body. Indeed, the generation of impulses to reverse the bodily sways proved to demand less than half of the energy required to stabilise the standing posture with continuous controllers (Bottaro *et al.* 2008). Therefore, activation of the MG muscle in standing is event driven; it occurs chiefly on the initiation of forward sways. This state-dependent activation of motor units likely relates to the minimisation of unnecessary muscular effort in standing.

Intermittent recruitments at long intervals (>1 s) occurred on the initiation of forward sways, whereas short interval (~500 ms) recruitments occurred throughout individual, forward sways. What might be the origin of the recruitment of MG units at short intervals? When subjects stood at ease, their postural oscillations had a modal duration of ~2 s (N = 7 subjects; see also Loram *et al.* 2005). Thus, if the periodical motion of the standing body determines the rate of recruitment, we would expect the MG motor units to be recruited once every 2 s. Clearly, the average duration of postural sways does not account for the modal interval of 500 ms (Figure7*B*) between recruitments. Additionally, this modal interval is not congruent with the summed delay of sensory and motor transmissions observed in reflex responses (<100 ms; Nardone & Schieppati, 2004). There might, then, be an internal process constraining the rate of recruitment of the postural MG motor units. In manual tracking of unpredictable stimuli, for example, subjects control movements with a series of 2-3 ballistic actions per second (Craik, 1947). If issued at intervals shorter than half a second, successive controlling actions are disrupted and accuracy is thus lost. This temporal constraint was attributable to a psychological refractory period (Craik, 1947). Evidences of psychological refractoriness in standing firstly manifested as impulsive variations in the calf muscles length, with the soleus and MG muscles showing 2-3 shortenings per second (Loram *et al.* 2005). The rate of 2-3 actions per second was also observed in the manual control of an inverted pendulum and remained invariant as the load time constant was systematically altered from 0.5 s to 1.02 s (Loram *et al.* 2006). Here, we show that the ubiquity of two actions

per second extends to the level of motor units in the MG muscle. The rate of two recruitments per second is consistent with the existence of a psychological refractory period (Craik, 1947) and strengthen the notion put forth recently that stabilisation of standing posture involves an intrinsic, higher level planning process (Loram *et al.* 2005, 2006, 2011).

Although the intermittent recruitment of postural motor units seems to be subjected to the psychological refractory period, it is unlikely associated with performance. By tapping gently on a joystick, subjects do succeed in stabilising a virtual second order load with median intervals between successive taps ranging from 0.4 to 1.2 s (Loram *et al.* 2011). They adopt the modal rate of two actions per second only when pushed to prioritise performance (i.e., they focus on minimising load velocity or variations in load position; Loram *et al.* 2011). Performance, however, is not critical in standing, unless subjects are asked to minimise their sways (Loram & Lakie, 2002b). The triggering of units at lower or higher rates in standing, then, is possibly constrained by the time constant of the falling body and the period of psychological refractoriness, respectively. Delays introduced by successive recruitments at low rates (lower than 2 actions per second) would overtly hinder stabilisation. Conversely, recruitments triggered at high rates (higher than 2-3 actions per second) would not provide the control system with sufficient time for planning and executing actions (Loram *et al.* 2011). The modal interval of 500 ms between recruitments of MG units is not only evidence of an intrinsic temporal process which plans and thus triggers impulsive actions in standing (Loram *et al.* 2005, 2006, 2009, 2011). It also reflects an efficient, intermittent controller of the postural sways, which is more economical and more tolerant to precision than continuous controllers (Asai *et al.* 2009; Bottaro *et al.* 2008; Loram *et al.* 2011).

Which physiological mechanism might account for the intermittency of postural units?
While motoneurones appear to be differentiated in phasic and tonic cells (Bradac *et al.* 1997; Granit *et al.* 1956), the intermittent recruitment of motor units observed in this study is unlikely due to their intrinsic properties. Phasic motor units, which are also large motor units (i.e., type FF units; Burke, 1968), are those not able to discharge continuously for long periods; they discharge rather shortly. The intermittent behaviour

of these phasic units is governed inherently by their low oxidative metabolism (Bradac *et al.* 1997). Postural motor units, however, are small, highly excitable units and, thus, capable of discharging tonically (Burke, 1968). The intermittency of the postural MG motor units, then, is not a consequence of their physiological inability to sustain firing. There is not a physiological constraint limiting the most excitable units to be activated at periodical intervals as short as 500 ms. Even the homogeneous, red soleus muscles, for example, acts phasically in vertical jumps (Walmsley *et al.* 1978). Therefore, the intermittent recruitment of postural motor units in the MG muscle likely reflect the control of an unstable body with a series of discrete, ballistic-like actions, rather than their intrinsic properties.

Intermittent activity of motor units is similarly not attributable to synaptic noise. Motor units discharge either tonically or phasically, depending on how much their recruitment threshold and the target force differs. Typically, a difference of 10% of the maximal muscle force is sufficient for a tonically active unit to discharge intermittent trains of potentials (Riley *et al.* 2008). Random fluctuations in the synaptic input impinging upon the ventral horn cells usually accounts for the occasional occurrences of instantaneous discharge rate below physiological levels (i.e., <4 pps; Person & Kudina, 1971; Riley *et al.* 2008). The occurrences of low discharge rates observed for the postural MG motor units, however, were consistent and not occasional (compare our Figure 7*A* with the Figure 5 in Person & Kudina, 1971). This consistency reflects a genuine, physiological mechanism of intermittent, postural control of the human MG muscle.

Intermittent recruitment of postural MG units may be regulated by two physiological conditions: i) uniform distribution of synaptic input to a set of motoneurones which have similar activation thresholds. In this case, small changes in synaptic input, not substantially affecting the discharge rate, would produce recruitment of many motoneurones; or ii) non-uniform distribution of synaptic input across the postural motoneurones. Recruitment may, then, occur by directing the input to some motoneurones without substantially increasing the input to already active motoneurones. Our results do not allow for ruling out one of these two possibilities. However, the second hypothesis is in agreement with the observation that localised

stretching of the cat gastrocnemius results in localised reflex responses (Eng & Hoffer, 1997). Providing that motor units have small territories with respect to the MG length (Vieira *et al*. 2011), it is in principle possible that recruitment occurs for motor units localised along different MG sections and that, in standing, control of the gastrocnemius muscles is due to the flexible activation of localised muscle units (Vieira *et al.* 2010).

Conclusion

The rate coding and the recruitment of motor units in the MG muscle were studied to identify which mechanism contributes most to the control of human standing posture. Our results indicate that postural activation of the MG muscle was accounted chiefly by the recruitment of motor units. Also, we have shown that recruitment depended on the body position and velocity, with motor units being firstly recruited only on the initiation of forward sways. This active silencing of motor units in the more backward positions indicates the minimisation of muscular effort in standing. Of particular physiological relevance was the fact that motor units were recruited intermittently, with a modal rate of two recruitments per second. Similar rates of actions have been consistently observed when subjects balanced an unstable load, either manually or using their calf muscles. The modal interval of 500 ms between successive recruitment of MG motor units advocates the notion of an internal, temporal process triggering impulsive muscle actions for the control of human standing posture.

References

Asai Y, Tasaka Y, Nomura K, Nomura T, Casadio M & Morasso P (2009). A model of postural control in quiet standing: robust compensation of delay-induced instability using intermittent activation of feedback control. *PLoS One* **4**:e6169.

Bigland B & Lippold OC (1954). Motor unit activity in the voluntary contraction of human muscle. *J Physiol* **125**, 322-335.

Bottaro A, Yasutake Y, Nomura T, Casadio M & Morasso P (2008). Bounded stability of the quiet standing posture: an intermittent control model. *Hum Mov Sci* **27**, 473-495.

Bradacs H, Cooper R, Msghina M & Atwood H (1997). Differential physiology and morphology of phasic and tonic motor axons in a crayfish limb extensor muscle. *J Exp Biol* **200**, 677-691.

Caron O, Faure B, Brenière Y (1997). Estimating the centre of gravity of the body on the basis of the centre of pressure in standing posture. *J Biomech* **30**, 1169-1171.

Casadio M, Morasso PG & Sanguineti V (2005). Direct measurement of ankle stiffness during quiet standing: implications for control modelling and clinical application. *Gait Posture* **21**, 410-424.

Craik KJW (1947). Theory of the human operator in control systems. I. The operator as an engineering system. *British Journal of Psychology* **xxxviii**, 56.

De Luca CJ, LeFever RS, McCue MP & Xenakis AP (1982). Behaviour of human motor units in different muscles during linearly varying contractions. *J Physiol* **329**, 113-128.

Desmedt JE & Godaux E (1977). Ballistic contractions in man: characteristic recruitment pattern of single motor units of the tibialis anterior muscle. *J Physiol* **264**, 673-693.

Desmedt JE & Hainaut K (1968). Kinetics of myofilament activation in potentiated contraction: staircase phenomenon in human skeletal muscle. *Nature* **217**, 529-532.

Di Giuliohttp://jp.physoc.org/content/587/10/2399.abstract - target-1#target-1 I, Maganarishttp://jp.physoc.org/content/587/10/2399.abstract - target-1#target-1 CN, Baltzopouloshttp://jp.physoc.org/content/587/10/2399.abstract - target-1#target-1 V & Loram ID (2009). The proprioceptive and agonist roles of gastrocnemius, soleus and tibialis anterior muscles in maintaining human upright posture. *J Physiol* **587**, 2399-2416.

Eng JJ & Hoffer JA (1997). Regional variability of stretch reflex amplitude in the cat medial gastrocnemius muscle during a postural task. *J Neurophysiol* **78**, 1150-1154.

Feinstein B, Lindegard B, Nyman E & Wohlfart G (1955). Morphologic studies of motor units in normal human muscles. *Acta Anat* **23**, 127-142.

Fitzpatrick R & McCloskey DI (1994). Proprioceptive, visual and vestibular thresholds for the perception of sway during standing in humans. *J Physiol* **478**, 173-186.

Granit R, Phillips CG, Skoglund S, Steg G. Differentiation of tonic from phasic alpha ventral horn cells by stretch, pinna and crossed extensor reflexes. *J Neurophysiol* **20**, 470-481.

Hellebrandt FA (1938). Standing as a geotropic reflex. The mechanism of the asynchronous rotation of motor units. *American Journal of Physiology* **121**, 471-474.

Howell JN, Fuglevand AJ, Walsh ML & Bigland-Ritchie B (1995). Motor unit activity during isometric and concentric-eccentric contractions of the human first dorsal interosseus muscle. *J Neurophysiol* **74**, 901-904.

Jacono M, Casadio M, Morasso PG & Sanguineti V (2004). The sway-density curve and the underlying postural stabilization process. *Motor Control* **8**, 292-311.

Joseph J, Nightingale A & Williams PL (1955). A detailed study of the electric potentials recorded over some postural muscles while relaxed and standing. *J Physiol* **127**, 617-625.

Kato M, Murakami S & Yasuda K. Behavior of single motor units of human tibialis anterior muscle during voluntary shortening contraction under constant load torque. *Exp Neurol* **90**, 238-253.

Kennedy PM & Cresswell AG (2002). The effect of muscle length on motor-unit recruitment during isometric plantar flexion in humans. *Exp Brain Res* **137**, 58–64.

Lafond D, Duarte M & Prince F (2004). Comparison of three methods to estimate the center of mass during balance assessment. *J Biomech* **37**, 1421-1426.

Loram ID & Lakie M (2002a). Direct measurement of human ankle stiffness during quiet standing: the intrinsic mechanical stiffness is insufficient for stability. *J Physiol* **545**, 1041-1053.

Loram ID & Lakie M (2002b). Human balancing of an inverted pendulum: position control by small, ballistic-like, throw and catch movements. *J Physiol* **540**, 1111-1124.

Loram ID, Gawthrop PJ & Lakie M (2006). The frequency of human, manual adjustments in balancing an inverted pendulum is constrained by intrinsic physiological factors. *J Physiol* **577**, 417-432.

Loram ID, Gollee H, Lakie M & Gawthrop P (2011). Human control of an inverted pendulum: Is continuous control necessary? Is intermittent control effective? Is intermittent control physiological? *J Physiol* **589**, 307-324.

Loram ID, Lakie M & Gawthrop PJ (2009). Visual control of stable and unstable loads: what is the feedback delay and extent of linear time-invariant control? *J Physiol* **587**, 1343-1365.

Loram ID, Maganaris CN & Lakie M (2005). Human postural sway results from frequent, ballistic bias impulses by soleus and gastrocnemius. *J Physiol* **564**, 295-311.

Mariani J, Maton B & Bouisset S (1980). Force gradation and motor unit activity during voluntary movements in man. *Electroencephalogr Clin Neurophysiol* **48**, 573-582.

Masani K, Vette AH & Popovic MR (2006). Controlling balance during quiet standing: proportional and derivative controller generates preceding motor command to body sway position observed in experiments. *Gait Posture* **23**, 164-172.

Maurer C & Peterka RJ (2005). A new interpretation of spontaneous sway measures based on a simple model of human postural control. *J Neurophysiol* **93**, 189-200.

McGill KC, Lateva ZC & Marateb HR (2005). EMGLAB: an interactive EMG decomposition program. *J Neurosci Methods*. **149**, 121-33.

Nardone A & Schieppati M (2004). Group II spindle fibres and afferent control of stance. Clues from diabetic neuropathy. *Clin Neurophysiol* **115**, 779-789.

Person RS & Kudina LP. Discharge frequency and discharge pattern of human motor units during voluntary contraction of muscle. *Electroencephalogr Clin Neurophysiol* **32**, 471-83.

Peterka RJ (2002). Sensorimotor integration in human postural control. *J Neurophysiol* **88**, 1097-1118.

Riley ZA, Maerz AH, Litsey JC & Enoka RM (2008). Motor unit recruitment in human biceps brachii during sustained voluntary contractions. *J Physiol* **586**, 2183-2193.

Tax AA, Denier van der Gon JJ, Gielen CC & van den Tempel CM (1989). Differences in the activation of m. biceps brachii in the control of slow isotonic movements and isometric contractions. *Exp Brain Res* **76**, 55-63.

Theeuwen M, Gielen CC, Miller LE & Doorenbosch C (1994). The relation between the direction dependence of electromyographic amplitude and motor unit recruitment thresholds during isometric contractions. *Exp Brain Res* **98**, 488-500.

van der Kooij H & de Vlugt E (2007). Postural responses evoked by platform pertubations are dominated by continuous feedback. *J Neurophysiol* **98**, 730-743.

Vieira TMM, Loram ID, Muceli S, Merletti R & Farina D (2011). Postural activation of the human medial gastrocnemius muscle: are the muscle units spatially localised? *J Physiol* **589**, 431-443.

Vieira TMM, Windhorst U & Merletti R (2010). Is the stabilization of quiet upright stance in humans driven by synchronized modulations of the activity of medial and lateral gastrocnemius muscles? *J Appl Physiol* **108**, 85-97.

Walmsley B, Hodgson JA & Burke RE (1978). Forces produced by medial gastrocnemius and soleus muscles during locomotion in freely moving cats. *J Neurophysiol* **41**, 1203-1216.

Wuerker RB, McPhedran AM & Henneman E (1965). Properties of motor units in a heterogeneous pale muscle (m. gastrocnemius) of the cat. *J Neurophysiol* **28**, 85-99.

Figure Captions

Figure 1: Location of intramuscular electrodes

Pairs of wire electrodes were inserted at three locations in the medial gastrocnemius (MG) muscle. The most distal pair of electrodes was inserted at 10% ($MG_{90\%}$) of the distance d between the superficial extremity of the most distal MG fascicles (identified with ultrasound imaging; dashed line) and the popliteal crease. The distance between the location where the other two pairs of electrodes were inserted, with respect to the $MG_{90\%}$ location, equalled $0.15d$ ($MG_{75\%}$) and $0.30d$ ($MG_{60\%}$). Needles were inserted at approximately the same depth, thus ensuring that electrodes in different locations recorded from distinct muscle fascicles.

Figure 2: Firing patterns of motor units during quiet standing

A, shows the centre of pressure (CoP) and the centre of gravity (CoG), computed for the subject 1 during 35 s of quiet standing, and the three intramuscular EMGs. The firing pattern and the averaged action potential of the eight motor units identified in the three EMGs are shown in B. Vertical bars denote the instant of each motor unit discharge. Note that periods of motor unit activity coincides mostly with the time instants of shifts in the body CoG from a more backward to a more forward position.

Figure 3: Motor unit discharges in the phase plane

CoG velocity (ordinate) versus position (abscissa) is plotted for the instants in which 13 motor units of subject 1 (top), and 14 motor units of subject 7 (bottom), discharged during the 60 s of standing. Dashed lines mark zero velocity and zero position. Zero position means the average CoG position throughout standing. Motor units discharges do not distribute uniformly on the phase plane. They are rather concentrated in the first and fourth quadrants, where the transition between forward and backward sways occurs.

Figure 4: Reliance of motor unit discharges on body position and velocity

The mean number of cases (N = 7 subjects) where motor unit discharges were found to occur inside each of the eight percentiles, between 10% and 90%, are shown for the distribution of CoG velocity (top) and position (bottom). Whiskers correspond to

standard deviations. Number of cases was normalized by the total number of motor units identified for each subject. Fifty percent corresponds to zero velocity and zero (average) CoG position. Dashed lines correspond to regression lines, estimated with the method of the least square error.

Figure 5: Recruitment and discharge rate of motor units in the phase plane.

A, shows the average number of active motor units (N = 7 subjects) in the phase-plane. Contour lines denote regions in the phase-plane where the number of active units increased by one. *B,* shows a similar plot for the average discharge rate of individual, active motor units. Contour lines indicate an increase in the average discharge rate of 0.5 pps. The lighter the region in the phase-plane, the more motor units active and with a higher discharge rate.

Figure 6: Motor unit recruitment accounts for the stabilisation of body sways

The number of motor units active (top) and their average discharge rate (bottom) are plotted across the eight percentiles, from 10% to 90%, of the distribution of sway velocities (□) and sway positions (■). The Pearson correlation coefficients (*R*) and their *P* values are shown for each plot. Note that the relative increase in the number of active units with sway velocity and position is far higher than that in the average discharge rate of active units.

Figure 7: Intermittency of motor unit recruitment during standing.

A, shows the histogram of the instantaneous firing rate pooled for all motor units and all the seven subjects tested. Note that the distribution of instantaneous discharge times ranged from 0.5 to 13.5 pps, with one clear peak centred on ~9 pps and another less evident peak located from 2 to 3 pps. *B*, illustrates the distribution of the interval between successive recruitments of individual motor units, with a clear peak centred on 500 ms. *C*, shows the average number of occurrences of motor unit recruitment (N = 7 subjects) in the phase-plane. Brighter regions indicate more occurrences of recruitment. Contour lines denote regions in the phase-plane where the number of recruitment events increased by one.
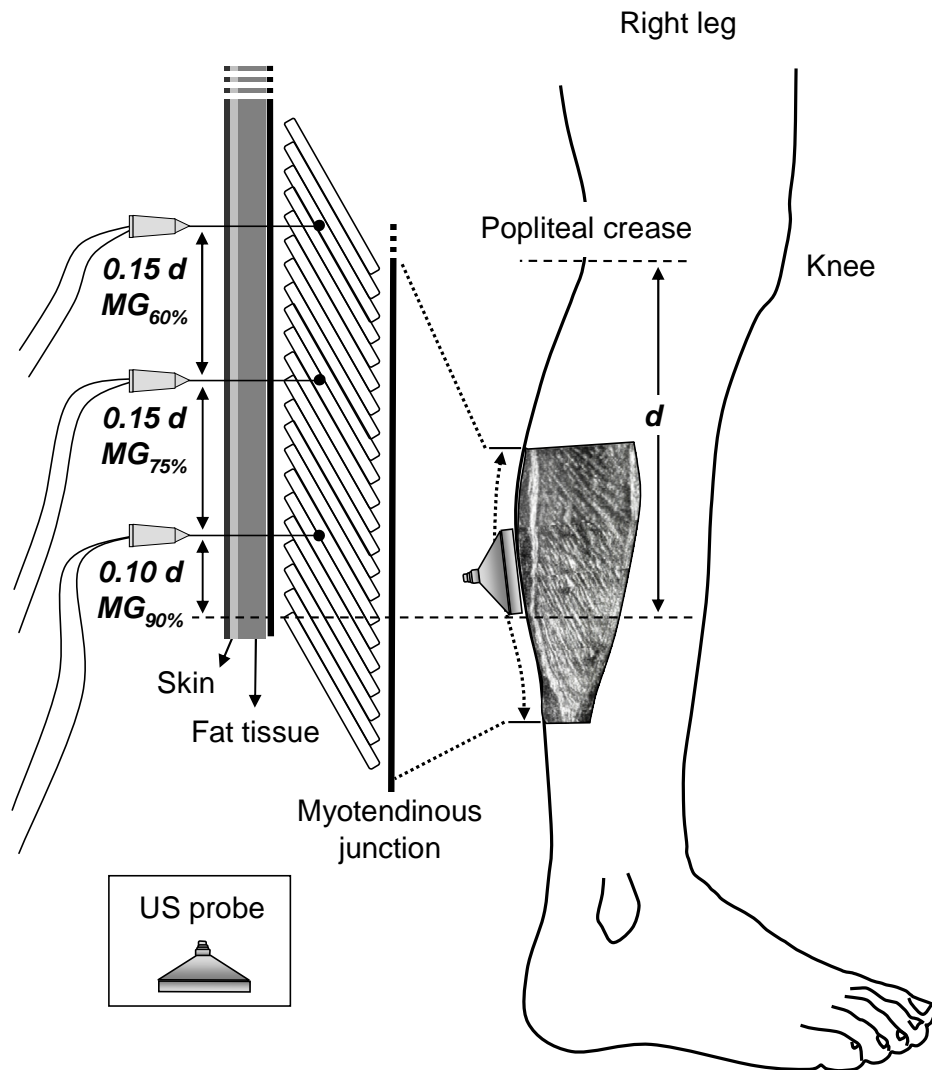
Figure 1

Figure 2

A

Stabilometric recording and intramuscular EMGs recorded from the three muscle locations

— CoP
— CoG

Position (mm)

5

0

-5

-10

$MG_{60\%}$

$MG_{75\%}$

$MG_{90\%}$

B

Firing pattern of individual motor units identified from the intramuscular EMGs
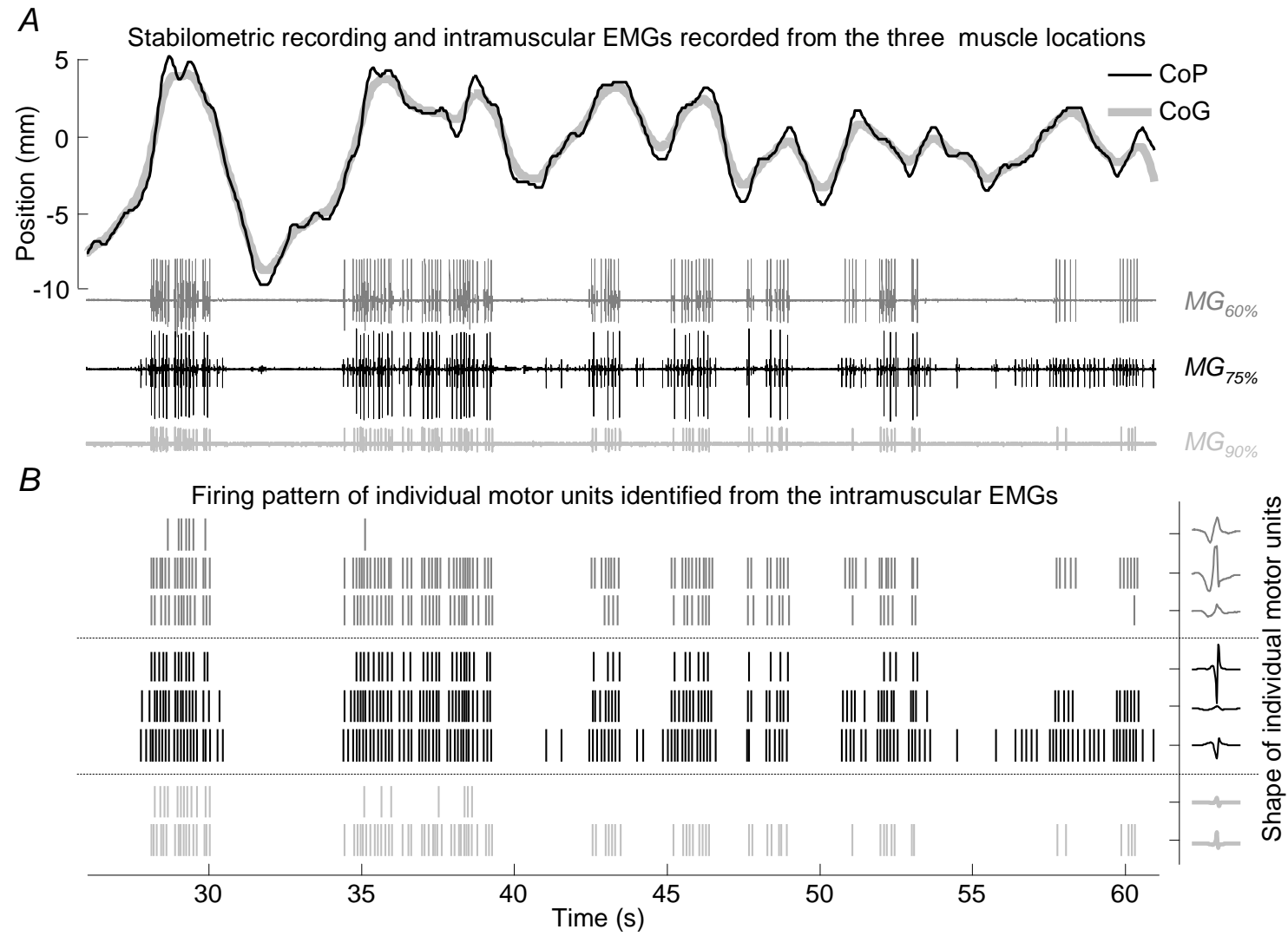
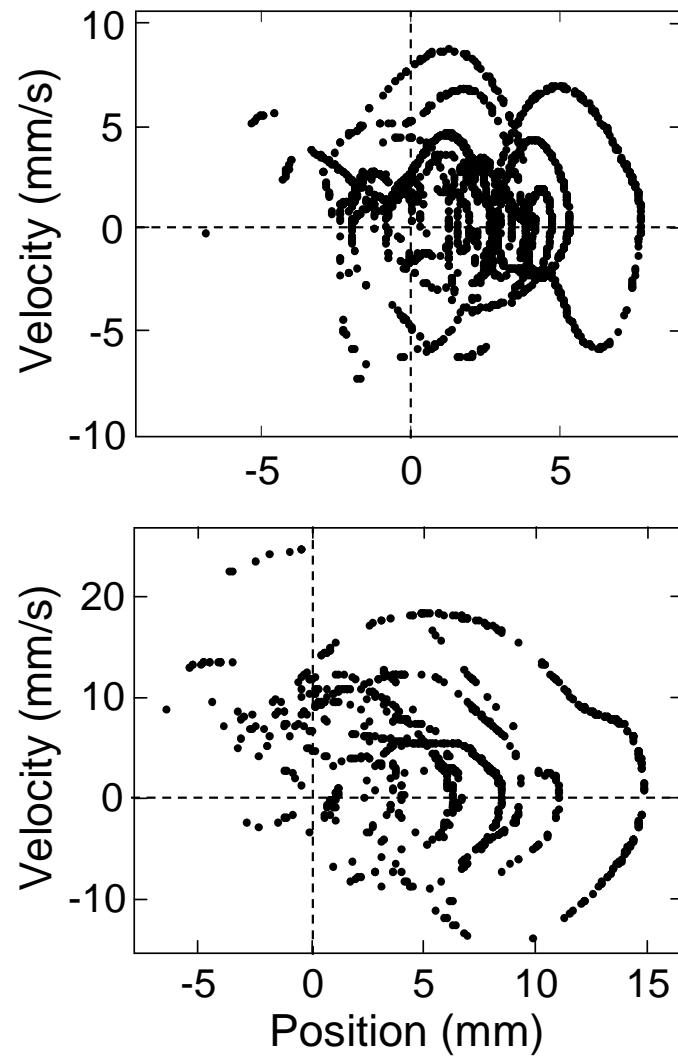Shape of individual motor units
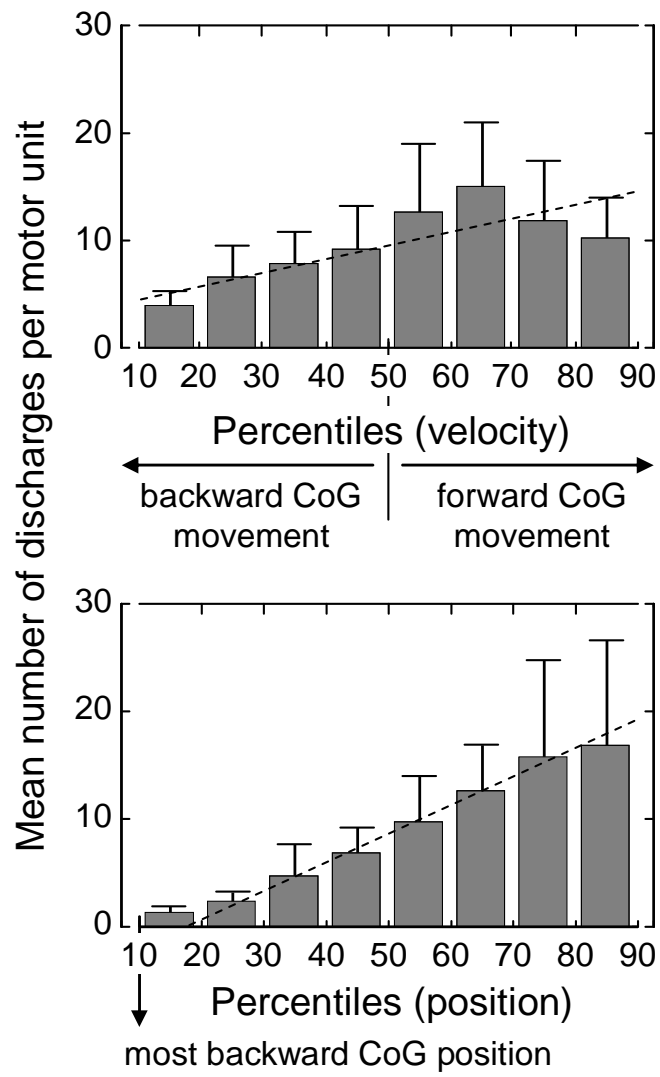
30   35   40   45   50   55   60

Time (s)

Figure 3

Figure 4

Figure 5

Figure 6

Figure 6

Top panel:

Number of motor units active (y-axis, 0–9)

- Sway velocity (open squares, dashed line)
- Sway position (filled squares, solid line)

$R = 0.27;$
$P < 0.001$

$R = 0.55;$
$P < 0.0001$

Bottom panel:

Instantaneous discharge rate (pps) (y-axis, 6–12)

--- $R = 0.22; P = 0.001$
— $R = 0.07; P = 0.20$

Percentiles (x-axis: 10 20 30 40 50 60 70 80 90)

33

Figure 7

A

B

C   average number of recruitment occurrences