Does the activity of ankle plantar flexors differ between limbs while healthy, young subjects stand at ease?

Elsevier Editorial System(tm) for Journal of Biomechanics

Manuscript Draft

Manuscript Number: BM-D-17-01164R2

Title: Does the activity of ankle plantar flexors differ between limbs while healthy, young subjects stand at ease?

Article Type: Short Communication (max 2000 words)

Keywords: postural control, electromyography, center of pressure, triceps surae, standing.

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Abstract: Inferences on the active contribution of plantar flexors to the stabilisation of human standing posture have been drawn from surface electromyograms (EMGs). Surface EMGs were however often detected unilaterally, presuming the myoelectric activity from muscles in a single leg reflects the pattern of muscle activation in both legs. In this study we question whether surface EMGs detected from plantar flexor muscles in both legs provide equal estimates of the duration of activity. Arrays of surface electrodes were used to collect EMGs from gastrocnemius and soleus muscles while twelve, young male participants stood at ease for 60 s. Muscles in each leg were deemed active whenever the Root Mean Square amplitude of EMGs (40ms epochs) detected by any channel in the arrays exceeded the noise level, defined from EMGs detected during rest. The Chi-Square statistics revealed significant differences in the relative number of active periods for both muscles in 10 out of 12 participants tested, ranging from 2% to 65% ($\chi2>17.90$; $P<0.01$). Pearson correlation analysis indicated side differences in the duration of gastrocnemius though not soleus activity were associated with the centre of pressure mean, lateral position ($R=0.60$; $P=0.035$). These results suggest therefore that surface EMGs may provide different estimates of the timing of plantar flexors' activity if collected unilaterally during standing and that asymmetric activation may be not necessarily associated with weight distribution between limbs. Depending on the body side from which EMGs are collected, the active contribution of plantar flexors to standing stabilization may be either under- or over-valued.

Dear Editor-in-chief

We respectfully submit to your attention the revised manuscript: "Does the activity of ankle plantar flexors differ between limbs while healthy, young subjects stand at ease?" This manuscript was submitted as a Short Communication and it contains new interpretations and relevant experimental information on how young, healthy individuals activate their plantar flexors during standing. Our manuscript has four figures and the word count is currently 2000.

In the revised manuscript we addressed the main concerns raised by reviewers. More specifically, the reviewers suggested some minor revisions to clarify our study. A list of points of how we have responded to the reviewers' suggestions was uploaded with the revised manuscript. We appreciate the comments raised by both reviewers and believe they assisted us in producing a stronger manuscript.

Other information: this manuscript is authored by FV dos Anjos, M Gazzoni and TM Vieira. The material within has not been and will not be submitted for publication elsewhere except as an abstract or as part of academic thesis. All the authors contributed substantially to all of the following areas indicated: i) the conception and design of the study, or acquisition of data, or analysis and interpretation of data; ii) drafting the article or revising it critically for important intellectual content; iii) final approval of the version to be submitted.

We look forward to your acknowledgment and we would like to thank the reviewers for their thoughtful comments about the manuscript. We thank you very much for your attention.

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RESPONSE TO REVIEWERS' COMMENTS

Manuscript: Does the activity of ankle plantar flexors differ between limbs while healthy, young subjects stand at ease?

Date: 13/09/2018

REVIEWER #1: The authors addressed all my concerns.

REVIEWER #2:

The authors have submitted a revised short communication on the comparison of calf muscle activation between left and right sides during standing.
I think this study is interesting and relevant. Many biomechanics studies assume symmetry particularly in healthy individuals. I think this study highlights that this is not necessarily the case. I think further work could be done for this manuscript.

We appreciate the reviewer for the comment. We are pleased to know our message has been well received and we believe the comments from both reviewers assisted us in producing a strong manuscript.

1) The authors only evaluate the task of standing with eyes open, this does not challenge the subjects balance, which may explain why the within-subject variation is large as small sways elicit comparatively large changes in muscle activation. Also this explains why the authors see a modest correlation to the CoP. Perhaps additional task to challenge the subjects balance could have been used, such as standing eyes closed, balance on single leg ect.

We thank the reviewer for the comment. Although we strongly value the importance of exploring the effect of additional standing tasks on muscle activity, inferences on the optimal neuromuscular mechanisms of posture control have been often drawn from the calf muscles' EMGs while subjects stand with eyes open. Thus, our focus was on side differences in the plantar flexors' activity during this reference, standing condition.

We would like to add that we are currently unable to identify the contribution of different sources of variability within subjects. We agree that a more demanding task would possibly lead to greater consistency of plantar flexors' activation within subjects. Nevertheless, given we are unsure on how much more demanding the standing task should be, we feel it would be too speculative to advance that more demanding variants of standing could reduce the variability within subjects. It should be noted though that this variability further aggravates the issue we raise in the manuscript; i.e., unilateral sampling may provide unrepresentative EMGs from the calf muscles during standing.

2) The authors have investigated the differences in activation timing during standing. However, the level of activation is not considered in the analysis and warrants identification in the discussion section as a limitation of the study.

We thank the reviewer for this suggestion. We slightly amended discussion to accommodate the reviewer suggestion (Lines 208-218).

"First, we would like to mention we assessed asymmetries in the timing of activity, although we acknowledge the importance of quantifying the degree of muscle activity during standing. By averaging the amplitude of EMGs across the whole standing duration, a biased indication on the degree of activity would be provided; low amplitude may not indicate low activation but e.g. longer inactive than active periods (Dos Anjos et al., 2017). Moreover, the timing of muscle activity has provided substantial contribution to our understanding of the human, postural control (Di Giulio et al., 2009; Laughton et al., 2003). Finally, it should be noted we were able to account for spatial differences in the timing of activity within plantar flexors with arrays of electrodes (Fig. 2; see also Dos Anjos et al., 2017), providing representative estimations of side differences in the timing of activity."

3) The result of activity identified in different regions of the muscle is particularly interesting, however is not explored in any detail in this manuscript.

We thank the reviewer for raising this point. In agreement with the reviewer suggestion, we added a brief statement on the importance of sampling EMGs with multiple electrodes from plantar flexors (Lines 208-218).

Title

Does the activity of ankle plantar flexors differ between limbs while healthy, young subjects stand at ease?

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Notes

- We are submitting this work as a Short communication.
- Word count (Introduction through Acknowledgments): 2.000.

Abstract

Inferences on the active contribution of plantar flexors to the stabilisation of human standing posture have been drawn from surface electromyograms (EMGs). Surface EMGs were however often detected unilaterally, presuming the myoelectric activity from muscles in a single leg reflects the pattern of muscle activation in both legs. In this study we question whether surface EMGs detected from plantar flexor muscles in both legs provide equal estimates of the duration of activity. Arrays of surface electrodes were used to collect EMGs from gastrocnemius and soleus muscles while twelve, young male participants stood at ease for 60 s. Muscles in each leg were deemed active whenever the Root Mean Square amplitude of EMGs (40ms epochs) detected by any channel in the arrays exceeded the noise level, defined from EMGs detected during rest. The Chi-Square statistics revealed significant differences in the relative number of active periods for both muscles in 10 out of 12 participants tested, ranging from 2% to 65% ($\chi^2>17.90$; $P<0.01$). Pearson correlation analysis indicated side differences in the duration of gastrocnemius though not soleus activity were associated with the centre of pressure mean, lateral position ($R=0.60$; $P=0.035$). These results suggest therefore that surface EMGs may provide different estimates of the timing of plantar flexors' activity if collected unilaterally during standing and that asymmetric activation may be not necessarily associated with weight distribution between limbs. Depending on the body side from which EMGs are collected, the active contribution of plantar flexors to standing stabilization may be either under- or over-valued.

Keywords: postural control, electromyography, center of pressure, triceps surae, standing.

1.Introduction

Insights into the neuromuscular mechanisms underpinning the control of human standing posture have been gained from surface electromyograms (EMGs; Di Giulio et al., 2009; Heroux et al., 2014; Gatev et al., 1999). While this evidence substantiates the potential relevance of surface electromyography, inferences on the neuromuscular determinants of posture control have been often drawn from EMGs collected from calf muscles in either left or right leg. Controversial results suggest however EMGs collected unilaterally may provide a biased indication on the postural activation of plantar flexors. For example, although Masani et al. (2013) reported the left and right plantar flexors are activated equally during standing, others detected EMGs with different amplitudes between legs (Liang et al., 2016; Mochizuki et al., 2007). These controversies may lie in the local, postural activation of ankle extensors (Hodson-Tole et al., 2013; Vieira et al., 2011). It seems therefore relevant to ask whether inferences on the activation of plantar flexors may be drawn from EMGs collected unilaterally during standing.

In this study we specifically ask: are the left and right plantar flexors activated for equal durations during standing? Instances of muscle activation were estimated from multiple surface EMGs, providing a more representative view of activity of the whole muscle (Dos Anjos et al., 2017). To our knowledge this is the first study to systematically evaluate the bilateral representation of plantar flexors' myoelectric activity during standing.

2.Methods

2.1.Participants

Twelve male volunteers (range: 24-34years; 60-90kg; 1.70-1.87m) were recruited after providing written, informed consent. Experimental procedures conformed with the *Declaration of Helsinki* and were approved by the Local Ethics Committee.

2.2.Experimental protocol

Two different tasks were applied. First, subjects relaxed their muscles completely while in supine position. Surface EMGs collected at this condition were considered to set the background, noise level. Second, subjects stood barefoot on a force plate for 60 s, with eyes open, arms alongside the body and feet in a comfortable position (Fig. 1). Feet contours were drawn to ensure participants would keep the same stance throughout experiments. Subjects were engaged in active conversation to suppress any voluntary control of calf muscles' activity during standing (Loram and Lakie, 2002a). The second task was applied three times, with 2 min intervals in-between.

2.3.Data acquisition

Single-differential EMGs were collected from the soleus and medial gastrocnemius muscles of both legs with linear arrays electrodes. Ground reactions forces were sampled synchronously with EMGs. See Appendix for description on electrodes' positioning (Fig 1A) and centre of pressure (CoP) computation.

2.4. Assessment of muscle activity

Initially, EMGs were visually inspected for the identification of channels with contact problems or power line interference; 13 out of 432 channels were discarded. Specifically for gastrocnemius, the distal channels sampling from the same muscle fibres were excluded (cf. Fig. 1 in Hodson-Tole et al., 2013). EMGs were then band-pass filtered

(15–350Hz cut-off; 4th order Butterworth bidirectional filter) and the Root Mean Square (RMS) amplitude was computed over 40 ms epochs (Laughton et al., 2003), providing a total of 1,500 RMS values per channel.

The duration of muscle activity was estimated from RMS values. First, the background level was set as the mean plus three standard deviations calculated over 3s of rest (40 ms epochs; Laughton et al., 2003), ensuring minimal, if any, false positives (Di Fabio 1987). As multiple EMGs were collected from each muscle, the background level was defined separately for each channel. Muscles active-inactive states were therefore assigned for each channel and were processed through the logical disjunction ("Or") to provide a series of *active* instances; whenever the RMS amplitude of any channel in a given array exceeded the background level, the corresponding muscle was deemed *active* (cf. Fig. 2 in Dos Anjos et al., 2017). Finally, the duration of muscle activity for each leg was computed by counting the relative number of *active* periods during the whole standing test. The duration of muscle activity was averaged across the three standing tasks for statistical analysis.

2.5.Statistical Analysis

The Chi-square (χ^2; Dawson-Saunders and Trapp, 1994) test was applied separately for each muscle and subject to test for whether the proportion of *active* periods between limbs and muscles was similar during standing. Within-subjects variability was assessed with the coefficient of variation (CoV) of the absolute right-left differences in the proportion of active periods. After ensuring the data Gaussian distribution (Shapiro-Wilk's test, $P>0.23$), Pearson correlation was applied to verify whether asymmetries in the duration of activity (i.e., right/left ratio of the number of *active* periods) were associated with the CoP mean lateral position.

3.Results

3.1.Side differences in plantar flexors' activity

Activation periods obtained from multiple surface EMGs were not the same. As shown for a representative participant (Fig. 2A), gastrocnemius EMGs with relatively high amplitude were detected distally. Close inspection of these EMGs further indicates that action potentials of different motor units were detected from different regions, resulting in the identification of different periods of activity across channels. Our procedure for estimating the duration of activity was insensitive however to regional differences in EMG amplitude; regardless of where action potentials were detected they were considered to estimate periods of activity (cf. grey rectangles in Fig. 2B right panel).

Side differences in the duration of calf muscles' activity were observed for 10 out of 12 participants (Fig. 3). The absolute right-left difference in the duration of activity ranged from 3.7% to 65.3% for gastrocnemius (Fig. 3A; χ^2>33.35; P<0.01) and from 2.0% to 37.2% for soleus (Fig. 3B; χ^2>17.90; P<0.01). Differences in the duration of activity were not observed consistently for the same side and muscle; two and four participants activated respectively more frequently the right gastrocnemius and soleus muscles (circles and squares in Fig. 3). Although participants #5 and #6 activated the left and right gastrocnemius for a similar duration (~50%; Fig. 3A), both muscles were concurrently active during less than 30% of the time (grey rectangles in Fig. 3). Regardless of the leg considered, soleus was generally active for a longer duration than gastrocnemius (χ^2>4.19 and P<0.04 for 20 legs), except for participants #2 and #11 (left leg) and subjects #4 and #5 (right leg). Within-subjects variability was on average 49.7% (20.6%–80.9%; CoV median and inter-quartile interval) for gastrocnemius and

48.0% (21.1%–84.7%) for soleus, indicating relatively high and somewhat moderate repeatability of asymmetries in the duration of activity across trials.

3.2. Correlation between CoP lateral position and asymmetric activity

Associations between side-differences in *active* periods and CoP lateral position were muscle dependent. Subjects whose CoP was on average located closer to the right leg activated more frequently their right gastrocnemius (Fig. 4A). For soleus, no significant correlation between asymmetric activity and CoP lateral position was observed (Fig. 4B).

4. Discussion

Were plantar flexors active for similar durations between legs?

Individual results indicate gastrocnemius and soleus were active for different durations between legs. We analysed subjects separately because there was no reason to choose a grouping criterion. Our hypothesis that plantar flexors in both limbs would be activated for different durations during standing was based on side-differences in the amplitude of EMGs (Liang et al., 2016; Mochizuki et al., 2007). Even though subjects may alternate weight distribution between limbs (Blaszczyk et al., 2000; Haddad et al., 2011), we are not aware of any evidence suggesting subjects should activate consistently muscles in either leg. Indeed, our results show some subjects activated for longer durations the right plantar flexors whereas others showed the opposite (Fig. 3). Similarly, given the low and variable intrinsic ankle stiffness across subjects (Loram and Lakie, 2002b), there was no reason to expect different subjects would load equally muscle in either side and that such loading would persist across trials. If standing is the results of periodic, active compensations to unpredictable falls (Loram et al., 2005; Bottaro et al., 2005), with minimization of muscle activity being the primary goal of the postural control system (Kiemel et al., 2011), between and within-subjects variability is not surprising. This possibly explains both the: i) variable side differences in the duration of activity between subjects, ranging from 4% to 65% for gastrocnemius and from 2% to 40% for soleus; ii) large (~50%) CoV values across trials. Regardless of these inter and intra-individual differences in the duration of activity, asymmetries in the duration of gastrocnemius and soleus activity were generally observed (Fig. 3). Current results seem therefore to support the notion that muscles in both limbs were elicited for different durations during standing.

Side differences in the duration of activity differed between muscles (Fig. 3). Concerning gastrocnemius, the duration of activity was associated with CoP lateral position; subjects standing closer to the right leg activated for longer duration their right gastrocnemius (Fig. 4). This observation is consistent with the gastrocnemius contribution to ankle inversion torque (Lee and Piazza, 2008; Vieira et al., 2013). Similar reports for soleus were not found, possibly because its line of action is directed more closely to the midline of the foot than that of gastrocnemius (Lee and Piazza, 2008). Asymmetries in the timing of gastrocnemius' activity though not of soleus were partly explained (36%; Fig. 4) by lateral differences in CoP position, which may be associated with the uneven weight distribution between limbs (Genthon et al., 2008). Corroborating this differential muscle response, previous study observed the medial gastrocnemius responds to surface translations directed over a larger, oblique range than soleus (cf. Fig. 3 in Henry et al., 1998). When drawing considerations on differences between muscles from current results, it should be noted we sample EMGs from a small, medial soleus region (Fig. 1). As discussed by Agur et al. (2003), EMGs collected medially may reflect a predominant, plantar flexion action. Regardless of the actual, predominant action of the soleus region sampled here, asymmetries were observed (Fig. 3). Factors other than the uneven loading of both limbs may thus explain side differences in the duration of plantar flexors' activity. While the identification of these sources urges further investigation, current results suggest inferences on muscle activation during standing may not proceed from EMGs collected unilaterally.

What are the implications of asymmetric activation of plantar flexors?

First, we would like to mention we assessed asymmetries in the timing of activity, although we acknowledge the importance of quantifying the degree of muscle activity during standing. By averaging the amplitude of EMGs across the whole standing duration, a biased indication on the degree of activity would be provided; low amplitude may not indicate low activation but e.g. longer inactive than active periods (Dos Anjos et al., 2017). Moreover, the timing of muscle activity has provided substantial contribution to our understanding of the human, postural control (Di Giulio et al., 2009; Laughton et al., 2003). Finally, it should be noted we were able to account for spatial differences in the timing of activity within plantar flexors with arrays of electrodes (Fig. 2; see also Dos Anjos et al., 2017), providing representative estimations of side differences in the timing of activity.

Our results (Fig. 3) indicate that surface EMGs detected bilaterally do not provide equal estimates of the duration of plantar flexors' activity. Although these results are not in contrast with the view that humans sway as an inverted pendulum, the inverted pendulum assumption does not seem to justify stating ankle muscles in both legs are activated similarly during standing (Fig. 3). According to current results, the active participation of plantar flexors to the correction of bodily sways may be either under- or over-valued, depending on the body side from which EMGs are detected. Drawing inferences on the neural mechanisms governing the activation of plantar flexors during standing may therefore require the detection of EMGs from both legs.

Conflict of interest statement

There were no known conflicts of interest associated with this work.

Acknowledgements

This study was supported by a national research project funded by the Italian Ministry of Education, Universities and Research (Protocol number: 2010R277FT) and by Compagnia di San Paolo foundation; Fabio V. dos Anjos was recipient of a scholarship provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior / Ciência sem Fronteiras / Processo n◦ BEX 9404/13-9.

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Figure Captions

Figure 1. Electrodes and feet positioning.

A, shows the position of electrode arrays over the medial gastrocnemius and soleus muscles in both legs. A schematic illustration of feet positioning on the force-plate is shown in *B*. Foot length was calculated as the distance between the tip of the third metatarsal head and the calcaneus bone. The distance between the centers of the length of each foot was considered to define the lateral distance between feet and thus the anterior-posterior (AP_{axis}) and medio-lateral (ML_{axis}) axes.

Figure 2. Raw surface EMGs and *active* periods of plantar flexors.

A, example of single-differential EMGs recorded by channels 3, 5 and 9 from the left and the right medial gastrocnemius of a single, representative participant. *B*, shows an expanded view (500 ms; dashed area) of the raw EMGs shown in *A.* Grey rectangles indicate the *active* periods identified separately per channel and for all channels (grey rectangles shown below EMGs; cf. Methods). Note different channels detected different action potentials and therefore provided different *active* periods for the right gastrocnemius. Percentages denote the relative number of *active* periods (i.e., duration of muscle activity) throughout the whole (60 s) standing test.

Figure 3. Asymmetries in the duration of plantar flexors' activity.

The relative duration of activity of the left (circles) and right (squares) medial gastrocnemius (*A*) and soleus (*B*) muscles is shown for the 12 participants tested. Vertical, grey rectangles indicate the relative amount of the standing duration within which muscles in both legs were active concurrently. Asterisks indicate significant differences in the duration of activity between the legs (P<0.05).

Figure 4. Side differences in the duration of muscle activity and centre of pressure position.

Scatter plots are shown, with the ratio (right/left) of the duration of medial gastrocnemius (*A*) and soleus (*B*) activity plotted in the y axis and the centre of pressure (CoP) position in the frontal plane plotted in the x axis. CoP position was normalised with respect to the lateral distance between feet (cf. Fig. 1). Regression (dashed) lines were drawn for clarity.

Figure 1
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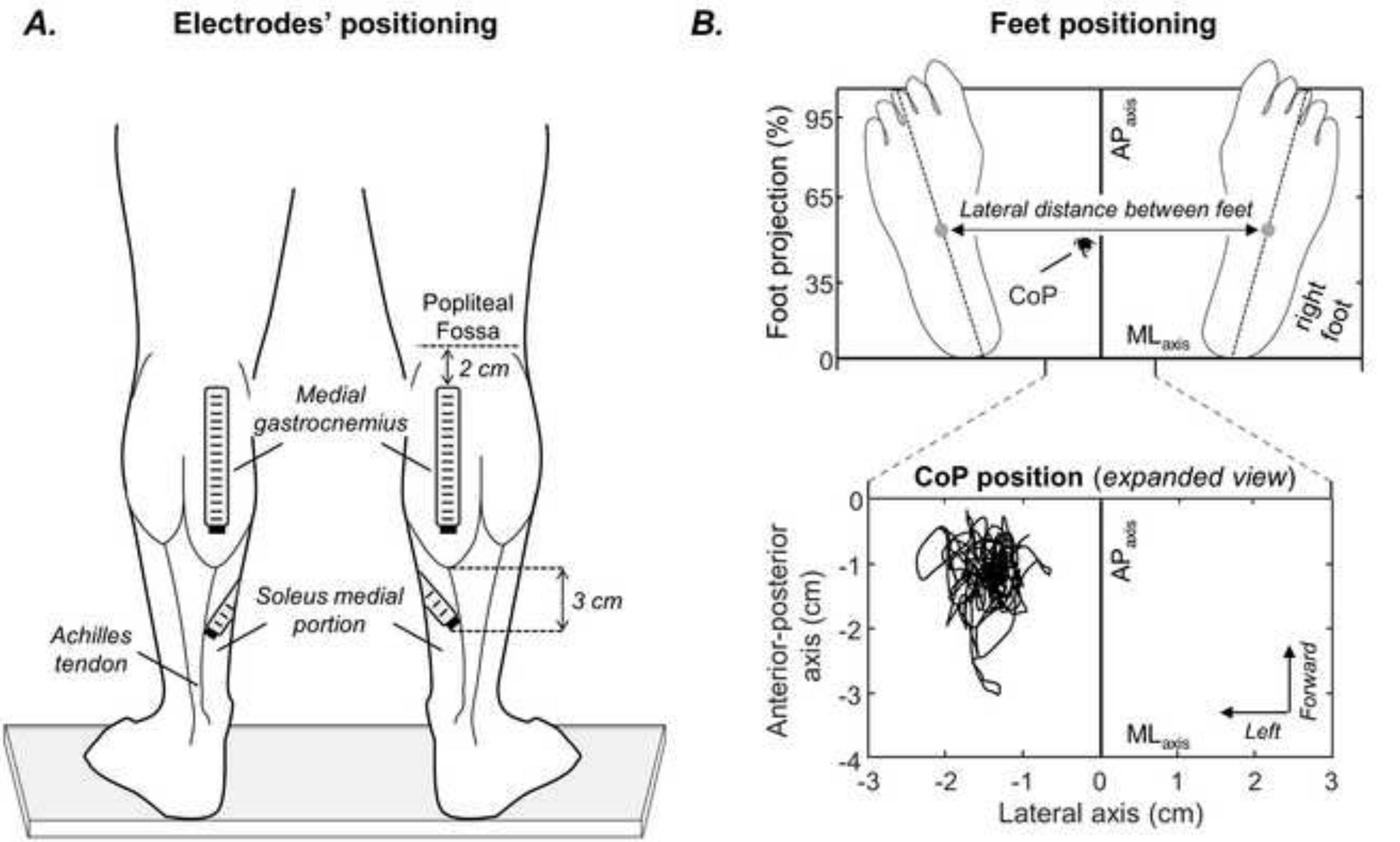

Figure 2

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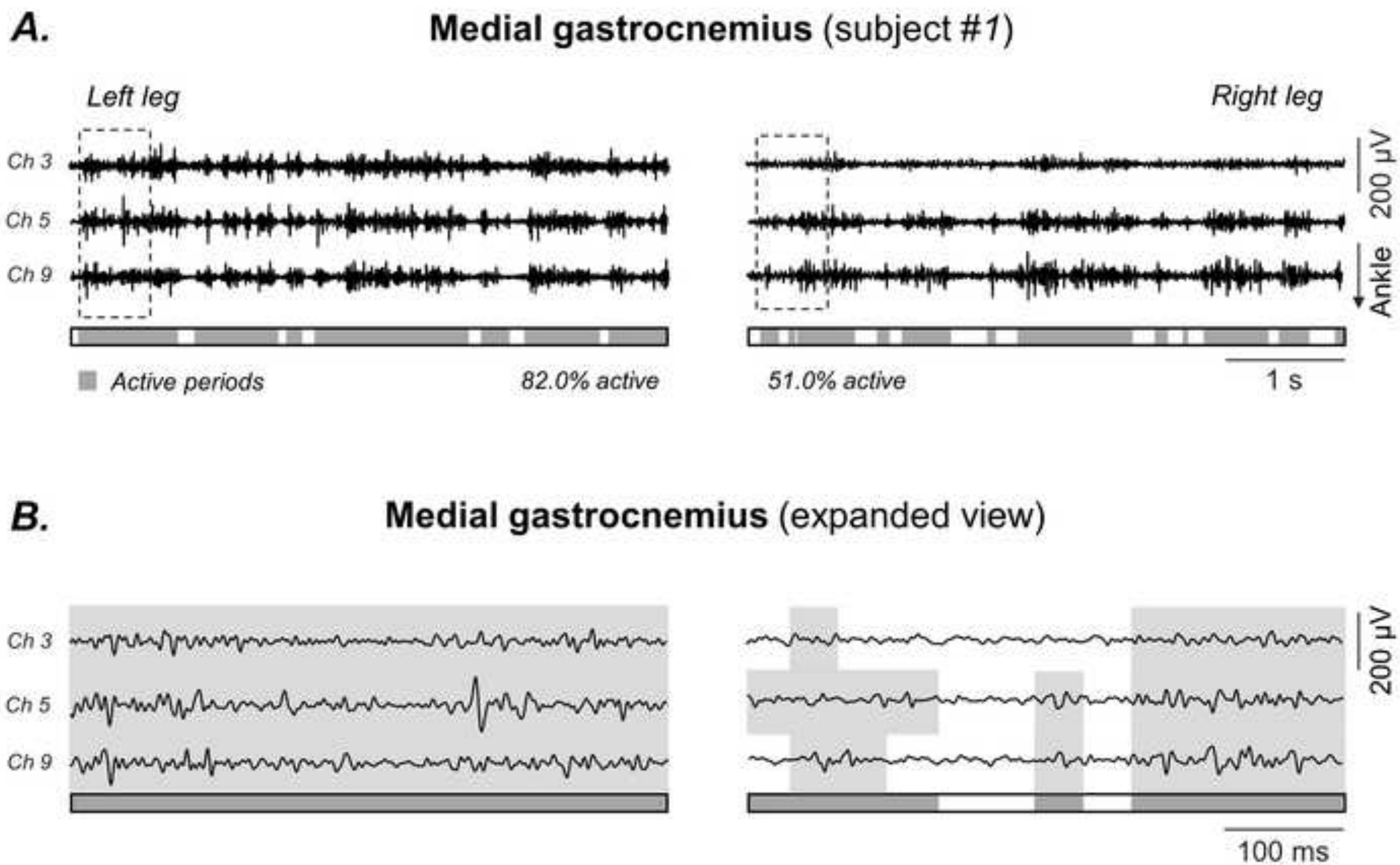

Figure 3

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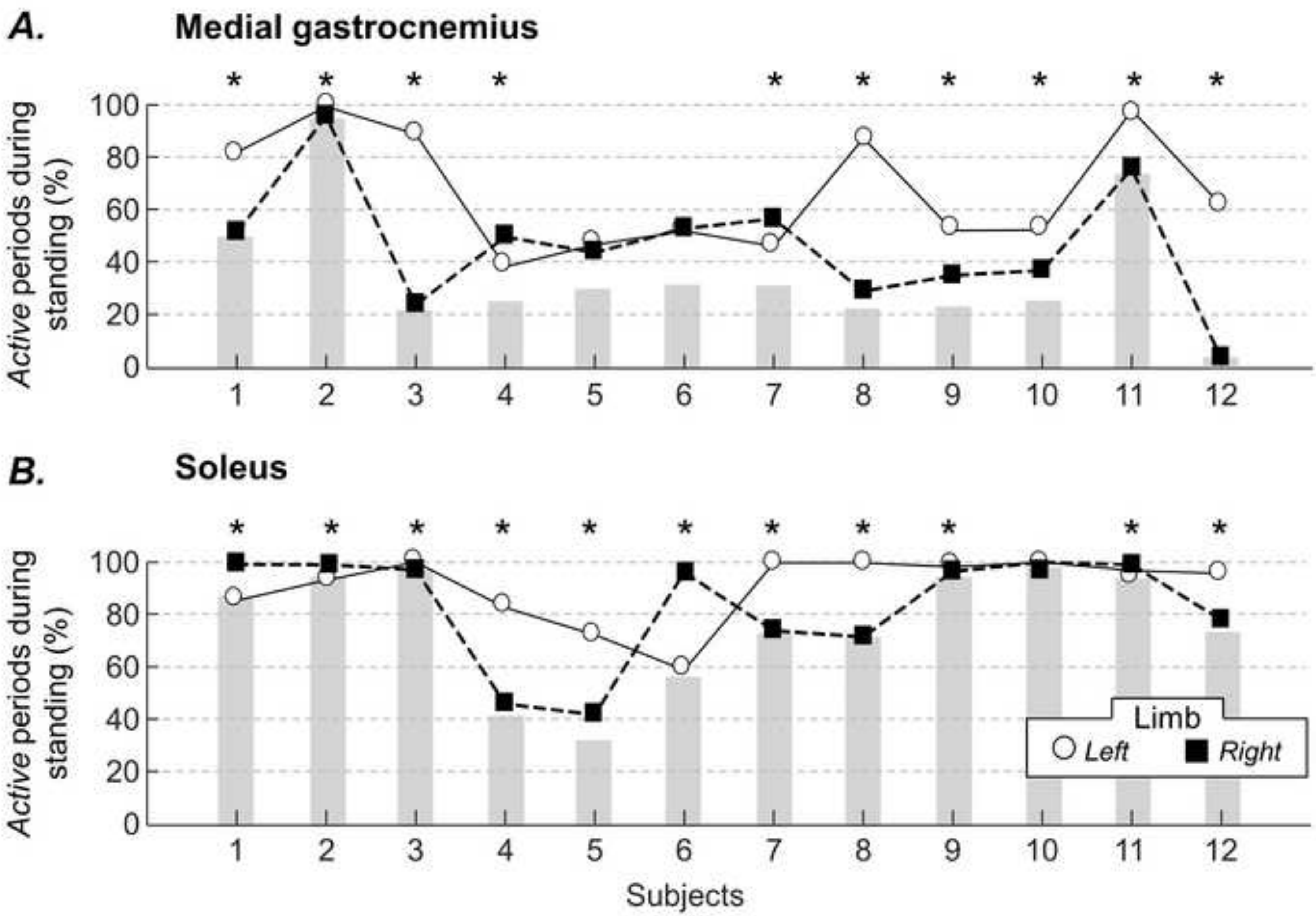

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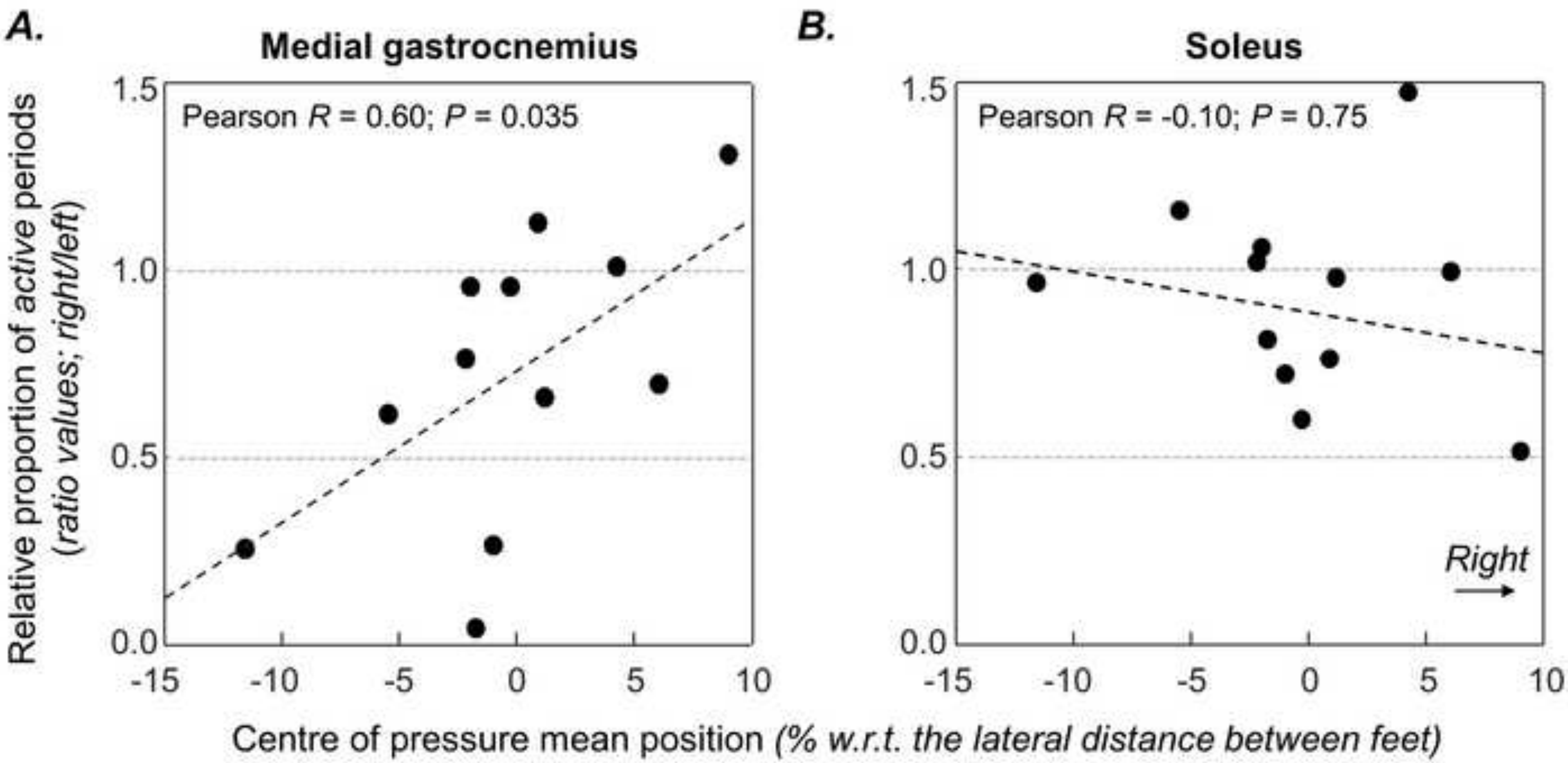

***Conflict of Interest Statement**

The authors declare that there were no known conflicts of interest associated with this work. The funders were not involved in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.