Corticospinal Excitability in Response to Mediolateral Gait Instability

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SUMMARY

Unpredictable gait disturbances, particularly in the mediolateral direction, pose a significant challenge to stability and are a common contributor to falls. Although the corticospinal tract is critical for gait and postural control, its response to such instabilities remains unclear. To investigate if corticospinal excitability increases during unpredictable lateral destabilisations , single-pulse transcranial magnetic stimulations were delivered over the primary motor cortex of 15 healthy individuals during steady-state and randomly laterally destabilised treadmill gait. Full-body kinematics were recorded using an optoelectronic motion capture system. Stimulations where the coil was >5 mm from the target location on the head were excluded. Corticospinal excitability was quantified for four upper- and three lower-leg muscles as the amplitude of the motor evoked potential (MEP) and compared between steady-state and destabilised gait. Lateral destabilisation resulted in a wider step width and shorter stride duration with increased variability and greater dynamic instability. Foot placement control during laterally destabilised gait was increased at midstance and there was also an increased average foot placement error in this condition. No differences in corticospinal excitability were observed in the lower-leg muscles. All upper-leg muscles demonstrated greater absolute MEPs in destabilised relative to steady-state gait. After normalising to muscle activity these periods became less pronounced, however, increases in corticospinal excitability during destabilised gait were observed in all but the gastrocnemius muscles. These finding suggest a heightened readiness of the corticospinal tract projecting to upper-leg muscles during destabilised gait, which could reflect general stabilising strategies such as decreasing stride time and increasing step width. Increases in the relative contribution of the corticospinal tract (i.e. normalised MEPs) were primarily noted during periods of low muscle activity, possibly hinting towards greater motor planning.

KEY WORDS:

Mediolateral gait stability, Motor cortex, Corticospinal excitability, Transcranial magnetic stimulation, Human gait

INTRODUCTION

Human gait is a complex task that requires coordinated activity of around 200 muscles. Despite the complexity of human gait, it can be seemingly effortless. Historically, it was assumed that this was achieved primarily through spinal control systems, a notion based on observations that quadrupedal mammals are capable of basic locomotion rhythmicity even in the absence of supraspinal processing [1–6]. While there are shared control mechanisms between human and quadrupedal gait [7,8], cortical lesions in humans can drastically debilitate gait [9,10] indicating an important role for supraspinal control. This likely reflects the greater stability demands of bipedal gait [11]. Understanding these supraspinal processes is essential, particularly for advancing fall prevention strategies and neurorehabilitation.

Transcranial magnetic stimulation (TMS) can be used to probe supraspinal processes. TMS of the motor cortex depolarises pyramidal tract cells and, with sufficient excitation, this results in the generation of action potentials that descend along the corticospinal tract to depolarise alpha motor neurones in the spinal cord [12–14]. The subsequent firing of motor units can be measured using surface electromyography (EMG) as a motor evoked potential (MEP). The size of the MEP reflects the excitability of the corticospinal tract [15,16].

Using TMS, it has been demonstrated that corticospinal excitability changes throughout the gait cycle, generally aligning with phasic muscle activity [17–19]. However, these excitability patterns do not always mirror ongoing muscle activity. Schubert and colleagues [20] observed increased excitability prior to phasic muscle activation and suggested that this reflects increased supraspinal input as an anticipatory adjustment aimed to preserve gait stability.

Gait stability is the ability to counteract the forces that induce moments to accelerate our centre of mass (CoM) away from the base of support, such as gravity and internal and external perturbations, by generating appropriate muscles forces, in order to remain upright [21–23].

Gait stability requires active control in the mediolateral direction [24,25], which is achieved through different strategies encompassing many muscles. Among these, the gluteus medius is often depicted as a primary contributor due to its important role in foot placement control [26–30]. Mediolateral gait stability control deteriorates with ageing [31,32] and is a strong predictor of fall risk [33,34]. Improving the understanding of how mediolateral gait stability is achieved will allow the development of strategies to prevent and reverse this deterioration, thereby reducing risk of falls.

During standing balance, a positive correlation between mediolateral sway velocity and the excitability of the corticospinal tract projecting to tibialis anterior has been reported [35]. However, less is known about how excitability of the corticospinal tract projecting to leg muscles is modulated during mediolaterally unstable gait, particularly when faced with unpredictable perturbations, such as missteps or lateral pushes in daily life. Since falls often result from such unpredictable disturbances, we test if corticospinal excitability increases in response to unpredictable mediolateral gait instabilities.

We hypothesise that corticospinal excitability will increase in accordance with mediolateral stability demands. Increases in corticospinal excitability will be observed throughout the gait cycle, reflecting heightened readiness to respond to unpredictable perturbations.

METHODS

Participants

Twenty-three participants (seven male, 16 females aged 18-40 years) provided written informed consent to participate. All participants reported that they were healthy adults without any contraindications to TMS or other risk factors that could impact the safety or reliability of this study (https://doi.org/10.17605/OSF.IO/7AJUZ and [36]). Participants were informed that they could withdraw from the experiment at any time. All procedures were conducted in

accordance with the Cardiff University School of Healthcare Sciences Research Ethics Committee. There were no adverse events. Six participants were excluded because no MEP could be observed within a comfortable stimulation intensity. One participant was excluded because they had to leave early. After processing, another participant was excluded because the TMS coil moved too much during the walking trials, resulting in less than 100 useful stimulations per condition. Data from the remaining 15 participants are reported here.

Procedures

The participants performed both steady-state and laterally destabilised treadmill gait within a Gait Realtime Analysis Interactive Lab (GRAIL) system (Motek Medical BV, Houten, Netherlands). Destabilisation was induced by continuous pseudorandom mediolateral oscillations of the treadmill with a peak-to-peak amplitude of 100 mm. The velocity of the treadmill was set at a constant speed of 4.0 km/h. Each condition (steady-state and laterally destabilised) consisted of a 200-s familiarisation period followed by a 20-min trial. The two conditions were performed in a single session with a break in between. The order of the conditions was randomised.

Motion capture

During the familiarisation and the trials, full-body kinematics were captured at 100 Hz using Vicon Nexus software (version 2.12, Oxford, UK). Spherical retroreflective markers (12-mm diameter) were placed in accordance with the full-body Plug-in Gait model (Vicon 2.12, Oxford, UK) with some modifications: the markers on the side/back of the head were placed on the side/back of the helmet (see below), and additional markers were placed on the nasal bridge and the left and right tragus.

For real-time detection of heel strike, the HBM 2.0 model (Motek Medical BV, *https://knowledge.motekmedical.com/wp-content/uploads/2019/07/HBM2-Reference-Manual-*

122 *Full-Body.pdf*) was used. Kinematic data were streamed into D-flow software (version 3.36,
123 Motek Medical BV) and processed in real time.

124 **EMG**

125 Before the trials, surface electrodes (Trigno Avanti Sensor, Delsys, Boston, MA, USA) were
126 placed on the following muscles on the right leg according to the SENIAM guidelines
127 (SENIAM): adductor magnus, biceps femoris, gluteus medius, rectus femoris, tibialis anterior,
128 gastrocnemius medialis, gastrocnemius lateralis. EMG data was collected at a sampling
129 frequency of 2000 Hz in Vicon Nexus software and was synchronised with kinematic data.

130 **TMS**

131 TMS (posterior-anterior current) was administered using a custom-made bent batwing coil
132 (Magstim, Whitland, Wales). The coil was positioned on the scalp over the left motor cortex
133 and position was adjusted until MEPs could be observed in the tibialis anterior of the right
134 leg. The position of the coil on the head of the participant was fixed using of a custom-
135 designed helmet (Figure 1). The weight of the coil and helmet was supported by two spring-
136 coil balancers that were attached the ceiling over the centre of the treadmill. The tension in the
137 spring-coil balancers was adjusted for each participant so that the coil rested on the scalp with
138 enough weight to ensure stability of coil position throughout the gait cycle (i.e., to prevent the
139 coil 'bouncing' with vertical head movement) but enough weight removed so that this would
140 not become uncomfortable over the duration of the experiment.

141 [insert figure 1]

142 For each participant, motor threshold was determined as the minimal stimulation intensity
143 required to evoke an MEP in tibialis anterior while the participant was standing quietly. This
144 was done according to the following procedure: First, the intensity of the stimulator was set to
145 35% of the maximal stimulation output, which (for most individuals) is a subthreshold

intensity within the limits of tolerability. Thereafter, the intensity was progressively increased in steps of 5% until a discernible MEP could be observed in at least 5 out of 10 stimulations, or the participant reported that they found the stimulation intolerable. If the latter occurred, the session was terminated (n = 6/23 participants). If the former occurred, the intensity was decreased in steps of 2% until the simulation did not evoke an MEP in 5 out of 10 stimulations. Lastly, the intensity was increased again in steps of 1% until the lowest intensity that consistently evoked a MEP was found.

During the experimental trials, the TMS intensity was set at 110% of motor threshold. Stimulations were given with random delays after heel strike with a varying number of strides between the stimulations (ranging from 3 to 5 strides). This resulted in approximately 200 stimulations over the 20-minute trial, evenly distributed across the stride cycle. The stimulation instant was recorded from the output of Dflow via a Phidget Interface Kit 8/8/8 (Phidgets Inc. Calgary, AB, Canada) with a Trigno Analog Input Adaptor (Delsys) that was sampled at 2000 Hz in Vicon Nexus software (version 2.12) synchronised with EMG data.

Relative coil displacement

Prior to the experiment, a static measurement was conducted using an additional marker placed at a central point within the helmet. During the experimental trials, the virtual marker's position was calculated using both the fixed helmet markers and the head markers. To determine the displacement of the helmet relative to the head, the deviation of the virtual marker calculated from the head markers was compared to its intended position, as defined by the rigid markers on the helmet.

Data processing

Gait events for offline analysis were calculated with a custom code where heel strike was identified as the moment the heel marker reach its lowest point and toe-off was identified as

the moment the heel marker reached its highest upwards acceleration (https://github.com/SjoerdBruijn/GaitEvents).

Gait pattern

The gait pattern in each condition was described using step width (average and variance), stride duration (average and variance), dynamic stability and quality of foot placement control in the last 150 strides of the 200-s familiarisation period.

Step width was defined as the mediolateral distance between the heels at heel strike. Stride duration was defined as the time interval between two consecutive right heel strikes.

Dynamic stability was calculated with the local divergence exponent (λ_s), a measure that quantifies the resilience to infinitesimal perturbations that occur during gait [37]. It is computed by determining the average exponential rate of divergence of neighbouring trajectories in state space [38]. Higher values indicate greater local instability. For these calculations, Rosenstein's algorithm was used [39]. The state space was created from time-normalised mediolateral velocity time series of the averaged thorax markers (C7 and left and right acromion) and five time-delayed copies. The λ_s was estimated as the slope of the mean divergence curve, with a normalised stride time from 0 to 0.5 stride [40,41].

The quality of foot placement control was calculated using the model of Wang and Srinivasan [28] (utilising codes from [42]).This model assumes a linear relation between the deviations in CoM state and subsequent foot placement (Equation below). Within our analysis the pelvis (PEL) was used instead of the CoM.

$$Foot\ Placement = \alpha(i) + \beta(i)_{position} * PEL(i)_{position} + \beta(i)_{velocity} * PEL(i)_{velocity} + \epsilon(i)$$

- $\beta_{position} = position\ regression\ coefficient$
- $\beta_{velocity} = velocity\ regression\ coefficient$

- $\epsilon = residual\ variance$ (i.e. foot placement error)

The foot placement was calculated in the single-leg stance phase, defined as the time between contralateral toe off and heel strike. To account for displacement, such as wandering backward, forward, left, or right, the coordinate system was taken from the previous stance foot. Additionally, within the laterally oscillating treadmill condition, the $PEL_{velocity}$ was corrected by adding the treadmill's lateral velocity to the derivative of the $PEL_{position}$. The degree of foot placement control was quantified by the relative explained variance (R^2). To attain normality in the data fisher transformation was performed on the R^2 values. Lastly, as the R^2 indicates the percentage of the foot placement variance explained by the variance in PEL states, we also calculated the average foot placement error as a measure of foot placement precision. This was calculated as the standard deviation of ϵ at the instant of foot placement.

Ongoing EMG and MEPs

EMG signals were bandpass filtered between 20 Hz and 500 Hz using a second-order bi-directional Butterworth filter. Ongoing EMG was calculated by rectifying and then averaging the filtered signal from 20 ms prior to stimulation to 10 ms after stimulation. The stimulation artifact was excluded by removing the data point at the time of stimulation (Figure 2). Absolute MEP amplitudes were calculated as the peak-to-peak amplitude of the filtered signal within the time window of 10 to 60 ms post-stimulation. Normalised MEPs were determined by dividing the absolute MEP by the ongoing EMG. MEPs were excluded if the relative helmet-head position at the time of stimulation was >5 mm along the vertical axis or >10 mm in the horizontal plane (combined displacement) from the original relative position. For each stimulation, the corresponding percentage of the gait cycle was determined based on the percentage of the total time between consecutive right heel strikes.

[insert figure 2]

For the ongoing EMG, non-normalised MEPs and normalised MEPs, all data points were weight based smoothed utilising SMART [43] with adjustments. First, all data points were linked to their corresponding gait cycle percentage (Figure 3A). Thereafter, the estimated time-series was reconstructed for each participant by convolving the kernel with the outcomes. This was then divided by the sum of the kernel density (weights) to maintain the proper scaling (Figure 3B & C). Lastly, normalised weights were calculated and averaged across participants for each time point in the gait cycle. This method ensures that participants with higher sample density at specific percentages of the gait cycle have a greater impact on the smoothed outcome at those corresponding time points.

[insert figure 3]

To accommodate the cyclical nature of the data, the SMART code was modified by replacing the standard Gaussian distribution, which assumes linear data, with the Von Mises distribution (Figure 4). To prevent bias by outliers, a smoothing kernel containing sufficient data points was required. For this at least six data points had to be contained within 2 standard deviations (σ) of each weight distribution (Figure 3B and 4). As the data displayed an average density of 1.99 ± 0.58/1% (steady-state) and 2.02 ± 0.55/1% (laterally destabilised) for each participant, a σ of 2% was chosen. Consequently, the Von Mises distribution with a kappa (κ) of 60, which contained the same amount of datapoints as a normal distribution with a σ of 2% (Figure 4A), was used.

[insert figure 4]

Statistics

Statistics were performed in Python (version 3.12.0). All kinematic outcomes, apart from the relative explained variance of the foot placement model, were compared between steady-state and laterally destabilised gait using paired t-statistics.

The relative explained variance (R^2) of the foot placement model over a step, ongoing EMG, non-normalised MEPs and normalised MEPs were compared between steady-state and laterally destabilised gait using cluster based-permutation testing according to the following procedure: First, t-tests were applied to each time point. However, since a difference at one time point is not independent of its neighbouring time points, condition differences were tested over clusters of consecutive time points, all of which exhibited statistically significant difference. This was done by summing the T values of a cluster and comparing this to summed T values of the permuted data's cluster (i.e. shuffling the data in a random order followed by smoothing it). Clusters were considered significant if their summed T value was greater than the 95th percentile of the 1000 permutations. This procedure was performed according to the code of Leeuwen and colleagues [43]. For all muscle activity and corticospinal excitability measures, this method was adjusted to account for the cyclic nature of the data, by wrapping the clusters around, connecting the clusters at the start and end of the gait cycle (https://doi.org/10.17605/OSF.IO/7AJUZ). For all statistical tests a significance threshold α of 0.05 was used.

RESULTS:

Of the 16 participants who completed the full experiment, one was excluded due to excessive relative movement between their head and the coil (i.e. fewer than 100 remaining stimulations per condition). Data are presented from the remaining 15 participants (four males and 11 females; age 18–40 years). Seven participants performed the laterally destabilised condition first and seven performed the steady-state condition first. Two participants received TMS stimulation at 100% of motor threshold instead of the targeted 110% due to reporting discomfort at the higher intensity. However, MEPs were still observed in these participants and their results are included.

Gait stability and quality of foot placement control

The lateral destabilisation evoked a significant increase in step width, step width variability and stride duration variability and a significant decrease in stride duration and the local dynamic stability, demonstrated by increased λ_s (Figure 5).

[insert figure 5]

The relative explained variance (Fisher-transformed R) of the foot placement model was greater in the laterally destabilised condition than in the steady-state condition at midstance. However, this difference was not observed in the rest of the stepcycle. Additionally, there was a significantly greater average foot placement error in the laterally destabilised condition (Figure 6).

[insert figure 6]

Ongoing EMG and MEPs

In total, more than 3100 stimulations (average 212 steady-state and 215 destabilised stimulations per participant) per condition were within the relative displacement threshold and included in analysis. This was 97.76% of the total stimulations. Steady-state and laterally destabilised gait were compared using a temporal resolution of 0.5% and a κ of 60. Our normalised weight distribution indicate standard deviations of sample spread of $7.7*10^{-4}$ and $4.9*10^{-4}$ for the steady-state and the laterally destabilised condition, respectively (Figure 7).

[insert figure 7]

Ongoing EMG

Compared to normal walking, destabilised walking increased muscle activity during some phase of the gait cycle in all muscles except the gastrocnemius medialis (Figure 8).

[insert figure 8]

Absolute MEPs

No differences in absolute MEP amplitude were detected in the lower-leg muscles (gastrocnemius medialis, gastrocnemius lateralis, and tibialis anterior). However, all upper-leg muscles demonstrated greater absolute MEPs during laterally destabilised gait than during steady-state walking (Figure 9). In the rectus femoris, MEP amplitude was larger over almost the entire gait cycle. A similar consistent increase in absolute MEPs was observed in the biceps femoris, with no observed differences during the terminal swing and a short period during the stance phase. The adductor magnus exhibited increased absolute MEPs from terminal swing into the early stance phase. In the gluteus medius, three periods of significantly increased absolute MEPs were observed: during early stance, from late stance into early swing, and during the late swing phase.

[insert figure 9]

Normalised MEPs

After normalising the absolute MEPs to the ongoing EMG, a short period of significantly increased MEPs during destabilised as compared to steady-state walking became apparent in the tibialis anterior, and increases remained significant in all upper-leg muscles (Figure 10).

In tibialis anterior, normalised MEP was greater during laterally destabilised gait than during steady-state walking during the transition from stance to the double support phase. In the rectus femoris, increases were noted during the stance and mid-swing phases. The biceps femoris demonstrated increased normalised MEPs during the stance phase and from double support into the mid-swing phase. The adductor magnus muscle exhibited two periods of significantly increased normalised MEPs: both during mid stance. Lastly, one period of increased normalised MEP was observed in the gluteus medius during the second double support phase.

[insert figure 10]

DISCUSSION:

We investigated whether unpredictable mediolateral gait instability was associated with greater excitability of the corticospinal tract to multiple leg muscles. For this purpose, we compared MEPs elicited during steady-state treadmill gait to those elicited during laterally destabilised treadmill gait. Our perturbation increased gait instability and caused participants to adopt a more cautious walking strategy. This was accompanied by increased activity of proximal and distal leg muscles and increased excitability of the corticospinal tract, particularly to proximal leg muscles.

Gait stability and quality of foot placement control

Similar to other studies [44,45], we observed a larger step width, step width variability, stride duration variability and shorter stride duration during laterally destabilised gait than during steady-state gait. Furthermore, we observed increased λ_s in the laterally destabilised condition, indicating poorer local dynamic stability, i.e. more susceptibility to small perturbations [46]. Increased variability in stride duration and step width is often associated with increased risk of falls [47]; however, it can also be attributed to a purposeful adjustment response to overcome the stability demands [11,44]. One of the main mechanisms behind this adjustment is foot placement control. While we did observe a tightening of this foot placement control during midstance, this did not result in a tightening of the actual foot placement (i.e. reduction in foot placement error at the end of the step cycle). The quality of foot placement control can increase due to a greater variance in the CoM_{states}, which most likely will happen due to the treadmill's oscillations. Thus, we also estimated the foot placement control error [48], and found that this increased during lateral destabilised gait (Figure 5), indicating a decreased precision in foot placement. This likely results from the constant nature of the perturbations,

as foot placement strategies require time to be executed [27,49,50]. Altogether participants widened their steps and increased stride frequency to maintain a stable gait in the presence of perturbations [51]. Increases in step width and reductions in step length are general strategies characterising “cautious walking” in unpredictable situations [51–53].

It is important to consider that the gait parameters measured during familiarisation might deviate from those during the actual trial due to the introduction of TMS. TMS itself already works as a central perturbation, leading to anticipation and compensatory adjustments for the evoked movements [54]. While both conditions should be equally affected by the additional stimulation, the gait patterns during the familiarisation might not perfectly align those during the trials.

Muscle activity and absolute MEPs

Muscle activity and absolute MEP amplitudes followed a similar phasic modulation over the gait cycle, which is in line with previous studies [17–19]. The cautious walking pattern employed in laterally destabilised gait was associated with greater activity of all muscles except gastrocnemius medialis. In the upper-leg muscles, this was mirrored by greater absolute MEPs. However, in lower-leg muscles there was no change in the amplitude of absolute MEPs. This was unexpected, but may be due to the method of destabilisation employed.

During steady-state gait, mediolateral stability is the dynamic equilibrium between the moments created by gravity and the forces generated by the muscles [55,56]. Primarily, the hip abductors, plantar flexors and to a lesser extent the dorsiflexors produce forces counteract the destabilisation due to gravity [22,55,57]. However, we suggest that in our laterally destabilised gait condition there was little demand on lower-leg muscles to compensate for the perturbations. Although lower-leg muscles play an important role in steady-state gait stability

[58] and ankle moments have the capacity to correct for preceding foot placement errors [59], the unpredictability of constant and random surface shifts during gait may have necessitated a greater reliance on the upper-leg muscles. This is reflected by the rectus femoris and bicepsfemoris demonstrating increases in MEP amplitude over a large portion of the gait cycle, which likely served as a proactive control strategy [15], aligning with the 'cautious walking' strategy. For the gluteus medius and adductor magnus the responses seem more specific, with the increases aligning the muscles contributions to mediolateral acceleration [55]. Therefore, we suggest that the method of destabilisation limited the applicability of lower-leg muscles strategies, forcing participants to rely on upper-leg muscles to maintain gait stability.

Normalised MEPs

The clear link between ongoing (pre-stimulus) EMG and absolute MEP amplitude is consistent with the established linear dependency between ongoing voluntary muscle activity and MEP amplitude [60]. However, this relation can become uncoupled if the corticospinal tract gets more (or less) access to the spinal motor neurone pool at a given level of muscle activity. Schubert and colleagues [20] reported early increases in tibialis anterior and gastrocnemius medialis MEPs prior to changes in phasic muscle activity during gait, which they interpreted as a preparatory mechanism to preserve gait stability. Although we observed some increases in normalised MEPs prior to the muscle activity (Figure 10: tibialis anterior from 50 to 60% , biceps femoris 70 to 85% and gluteus medius 95 to 5%), which were greater in laterally destabilised gait for the tibialis anterior and biceps femoris, this effect was not consistently observed across all muscles, nor was it amplified in the muscles known to be most involved in stabilising gait [11], such as the gluteus medius. Therefore, we suspect that early (normalised) MEP facilitation does not universally respond to (external) preparatory stability demands. Further research is required to understand why early facilitations occur.

Normalised MEPs were greater during destabilised gait than during steady-state gait in all upper-leg muscles and the TA. This indicates that the changes in excitability of the corticospinal tract were not solely driven by changes in muscle activity. Highlighting that excitability changes serve additional roles besides modulating the ongoing muscle activity [35].

No changes in normalised MEPs were observed across conditions for the gastrocnemii, this again likely reflects the limited use of ankle strategies to counteract our method of destabilisation [61,62]. The facilitation in normalised MEPs in the TA during the second double support phase may represent a preparatory effect to ensure toe clearance [63].The gluteus medius plays a predominant role in mediolateral gait stability yet there was only a short period around the second double support phase where normalised MEPs in this muscle were increased in the destabilised condition [11]. Although this is a transitional phase that does demand stabilisation [64], it does not reflect the phase with the greatest muscle potential to control this destabilisation, which is mostly seen as swing leg's midstance or the stance leg's late stance phase [11,50]. Similarly, the timepoints at which normalised MEPs were increased in the destabilised condition in the other muscles, although potentially functional, had no clear connection with specific stabilisation strategies [55,58]. This raises the question whether these changes are linked to stability demands, phasic postural adjustments or other factors.

We should take into account that our results could be affected by the inherent variability in both ongoing EMG and MEP signals. MEPs are subject to rapid, spontaneous fluctuations in corticospinal excitability, especially during periods of low excitability, which complicates their interpretation [65,66]. Additionally, during periods of low muscle activity, EMG signals become more susceptible to noise, further complicating the interpretation of normalised MEPs

[67]. Therefore, caution is advised in interpreting normalised MEPs, especially during periods of low excitability or muscle activity. Interestingly, regardless of the higher variability across stimuli within a condition during these phases of low muscle activity, most of the differences between conditions that we found were within periods of low excitability and muscle activity. This potentially indicates that the observed differences in normalised MEPs might be reflective of motor planning, typically occurring during pre-active states [68]. Such planning-related increases in MEP amplitude align with the idea that preparatory excitability reflects task complexity, even without concurrent increases in EMG activity [69].

Conclusions

In conclusion, we investigated the effects of unpredictable lateral destabilisation on the excitability of the corticospinal tract to multiple leg muscles. We found that the imposed perturbations increased instability and led participants to adopt a plethora of "cautious walking" strategies. This was associated with periods of increased absolute corticospinal excitability in all upper-leg muscles along with sustained increases in normalised MEPs, particularly at times of low muscle activity. These findings suggest that constant postural destabilisations heighten the responsiveness of the corticospinal tract to proximal leg muscles, suggesting that the corticospinal tract is involved in the adaptations to these conditions.

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Supplementary material

Opensource material of the raw data/codes and the questionnaire can be found at: https://doi.org/10.17605/OSF.IO/7AJUZ.

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Figures and legends

Figure 1

Figure 1. *The TMS-helmet with a built-in custom made bent batwing coil.*

Figure 2

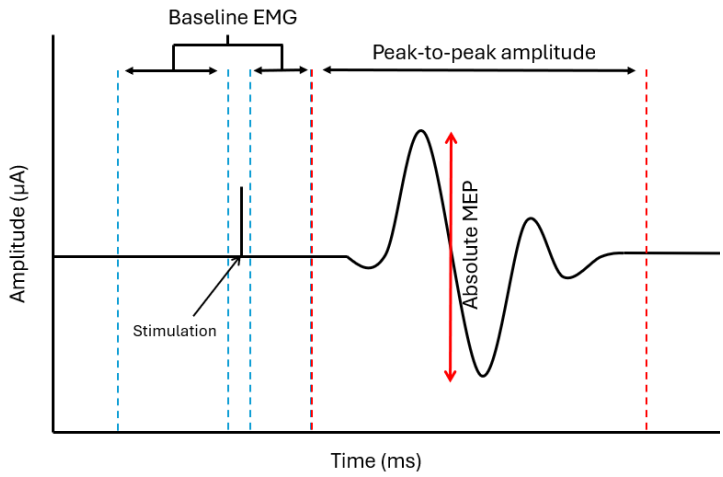

Figure 2. *Calculation of the ongoing EMG and absolute MEPs. The blue dotted lines indicate the windows over which the data was rectified and averaged to calculate the ongoing EMG. The red dotted lines indicate the window over which the peak-to-peak value was calculated indicating the absolute MEP*

Figure 3

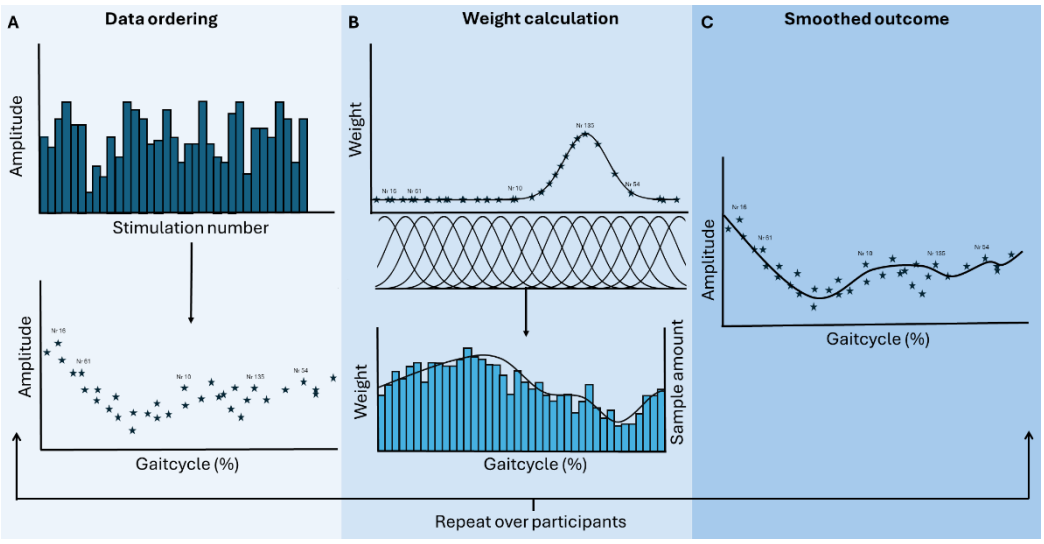

Figure 3. *Illustration of the process of weighted smoothing for data from one condition of one participant. A) Stimulations and how the number of stimulation was linked to their gait cycle percentage. B) Display of how for each time sample the weight was distributed. Repeating this over the new temporal axis set at a chosen temporal resolution results in a general weight distribution based on sample density. C) The smoothed results from convolving the weight distribution with the stimulation amplitude.*

Figure 4

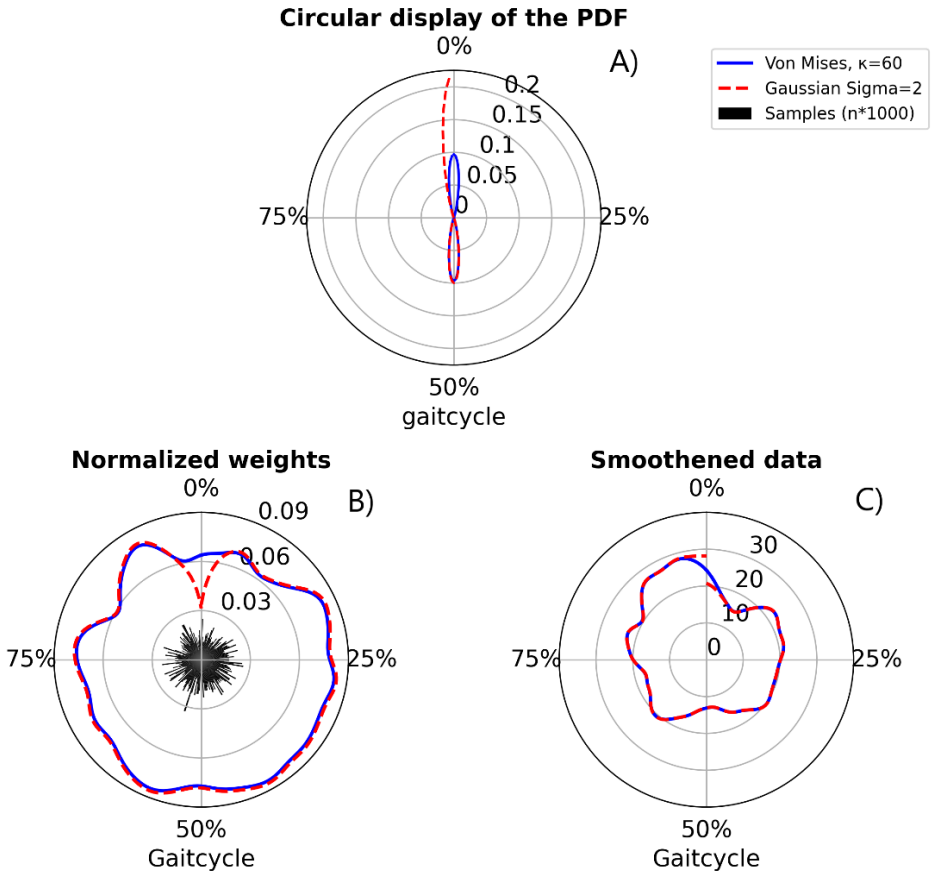

Figure 4. *Smoothing differences between the Von Mises and Gaussian distributions.* ***A)*** *Differences at timepoints 0 and 50.0% for the probability density function (PDF) of both the Von Mises and Gaussian distribution.* ***B)*** *Effect of sample density on temporal weights and how this differs between linear and circular distributions. The sample density is displayed in black bars with the amount of samples per 0.5%. The amount if equal to the y-axis times 1000* ***C)*** *Effect of the difference in weight distribution on the smoothed data outcome.*

Figure 5

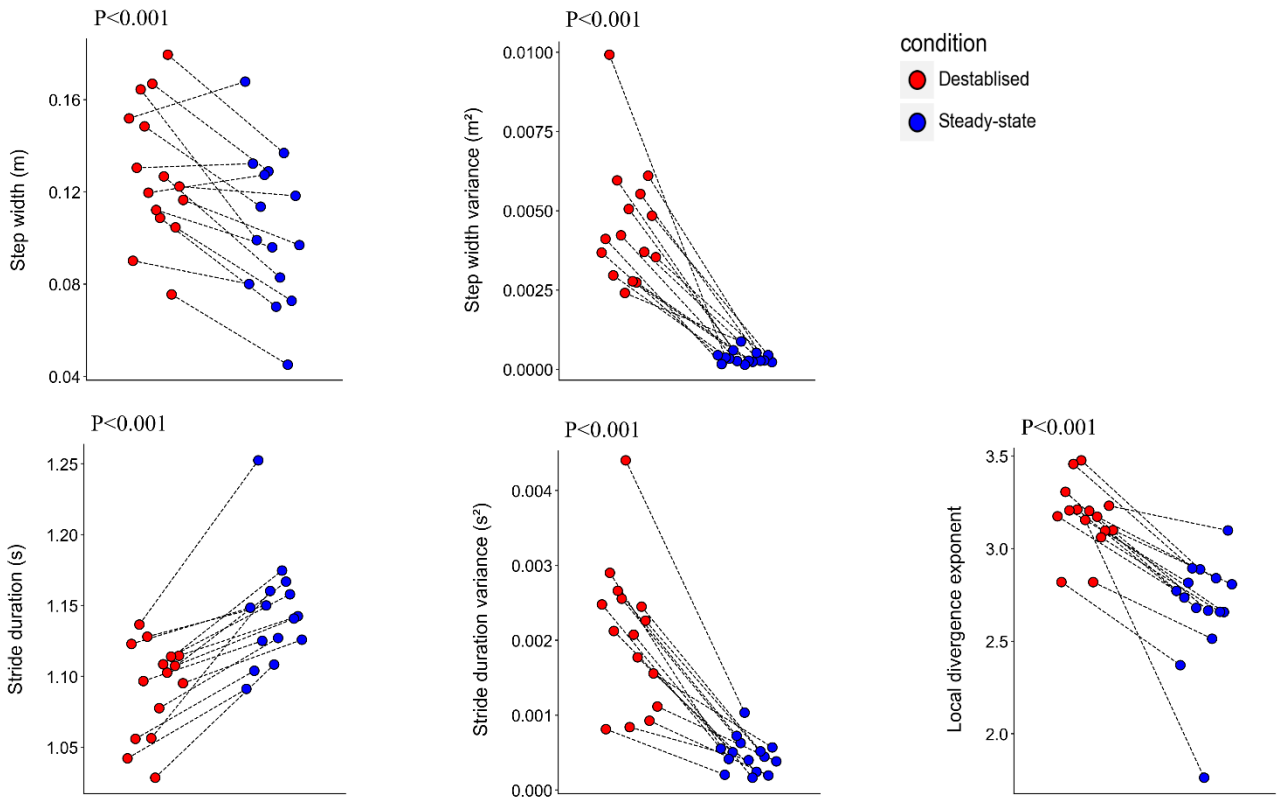

Figure 5. *The effect of lateral destabilisation on step width, step width variance, stride duration, stride duration variance, and the local divergence exponent (λs). The dots represent the outcomes of individual participants, connected between conditions by black dotted lines to show the changes for each participant.*

Figure 6

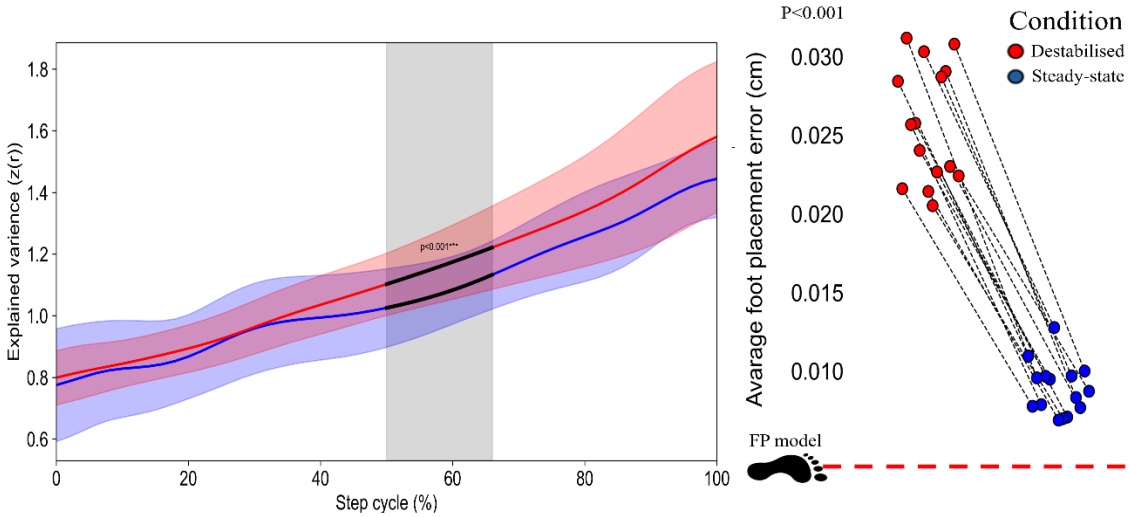

Figure 6. *A) fisher transformed explained variance of the foot placement as a function of step cycle. A step is defined from toe-off to subsequent heel strike. The coloured shaded area indicates the standard deviation of the corresponding condition. The black lines and grey shaded area indicate significant difference B) The average foot placement error (indicating how precise the actual foot placement is relative to the predicted foot placement) was increased during destabilised walking.*

Figure 7

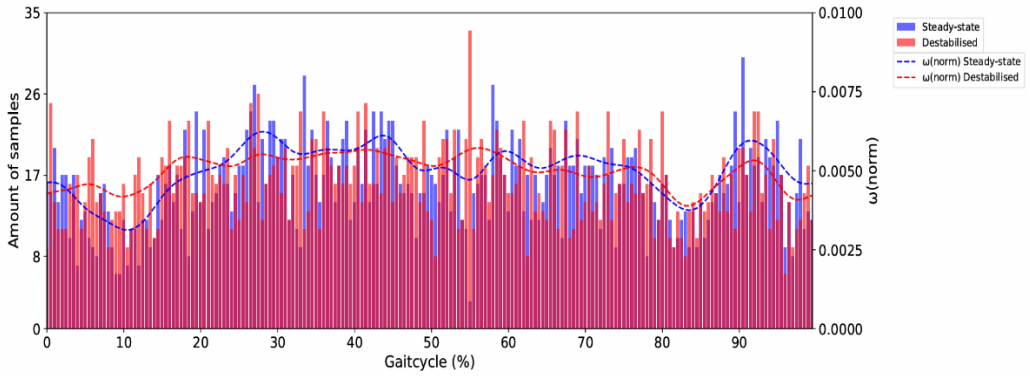

Figure 7. *The amount samples at each temporal point (i.e. the amount of samples per 0.5%) summed over all participants. Purple is seen due to the overlapping bars of both conditions. The dotted lines indicate the normalised weight at each timepoint. This was calculated based on the sample density, a perfect homogeneous data spread would result in a flat line at 0.0050.*

Figure 8

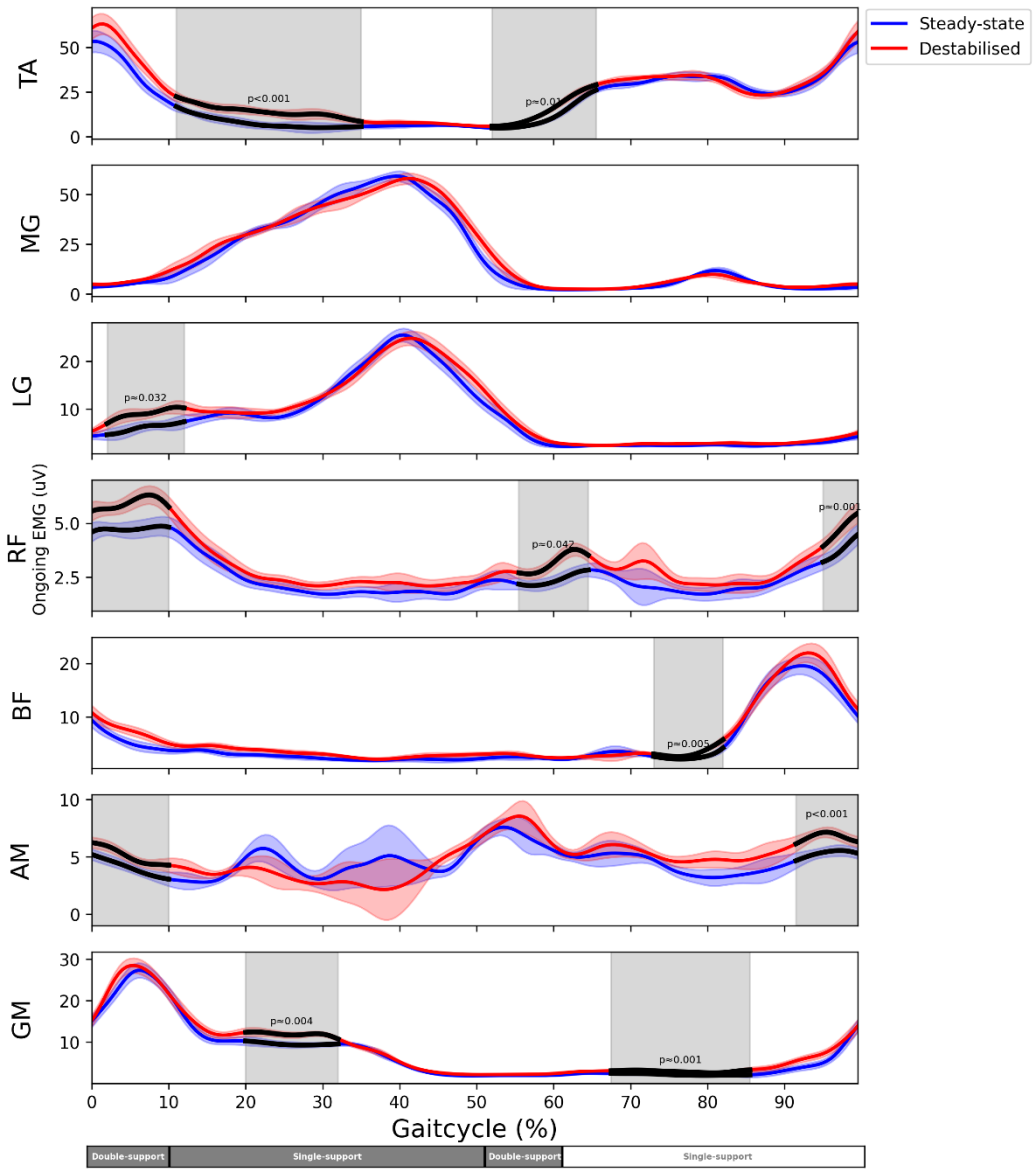

Figure 8. *Ongoing EMG's (uV) as function of the gait cycle for all muscles. 0% corresponds to right heel strike. The coloured shaded area's are the standard deviations of the conditions. The black lines and with the grey shade around it indicate significant difference. M. gastrocnemius medialis (MG), m. gastrocnemius lateralis (LG), m. rectus femoris (RF), m. biceps femoris (BF), m. adductor magnus (AM)*

Figure 9

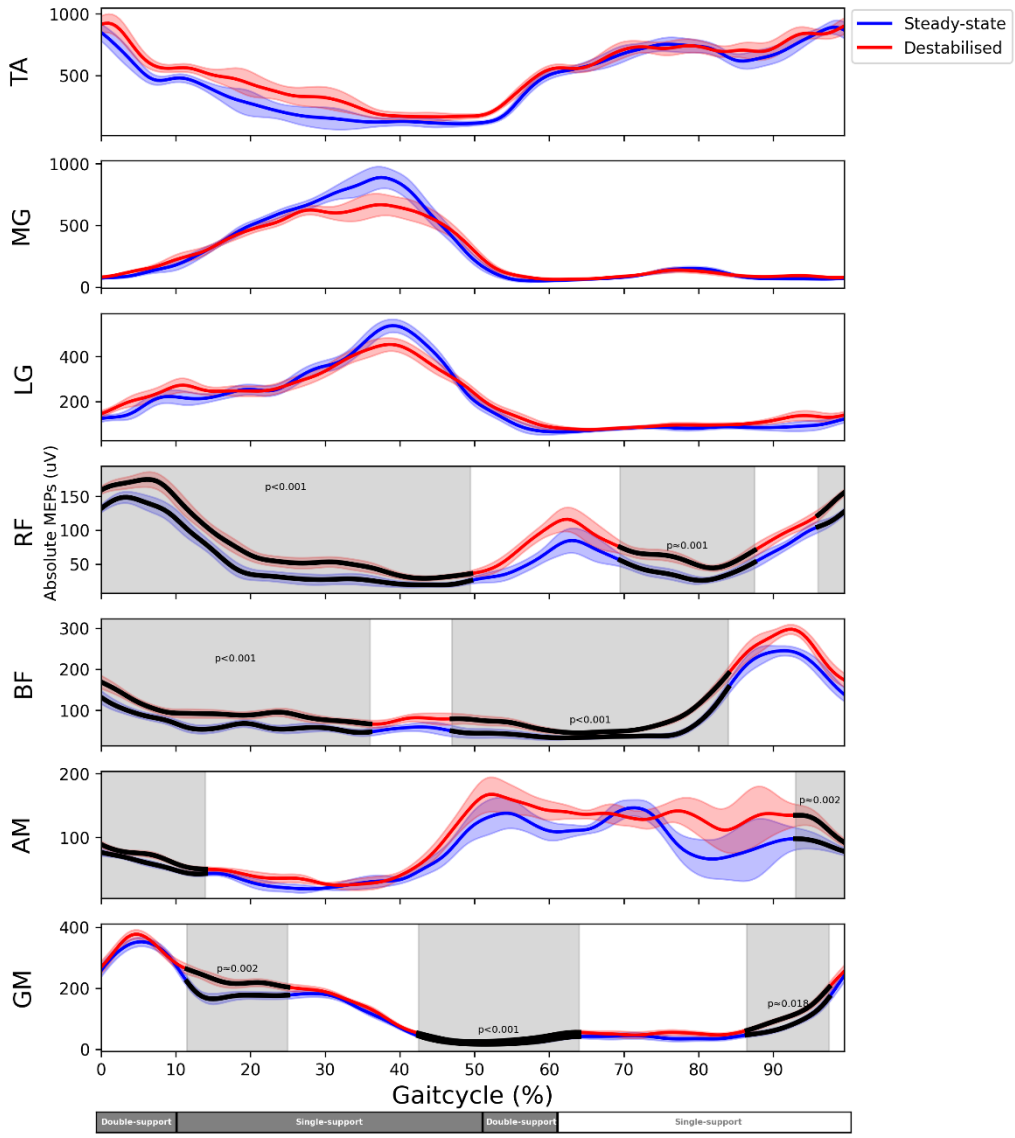

Figure 9. *Absolute MEPs(uV) as function the gait cycle for all muscles. 0% corresponds to right heel strike. The coloured shaded area's are the standard deviations of the conditions. The black lines and with the grey shade around it indicate significant difference. M. gastrocnemius medialis (MG), m. gastrocnemius lateralis (LG), m. rectus femoris (RF), m. biceps femoris (BF), m. adductor magnus (AM)*

Figure 10

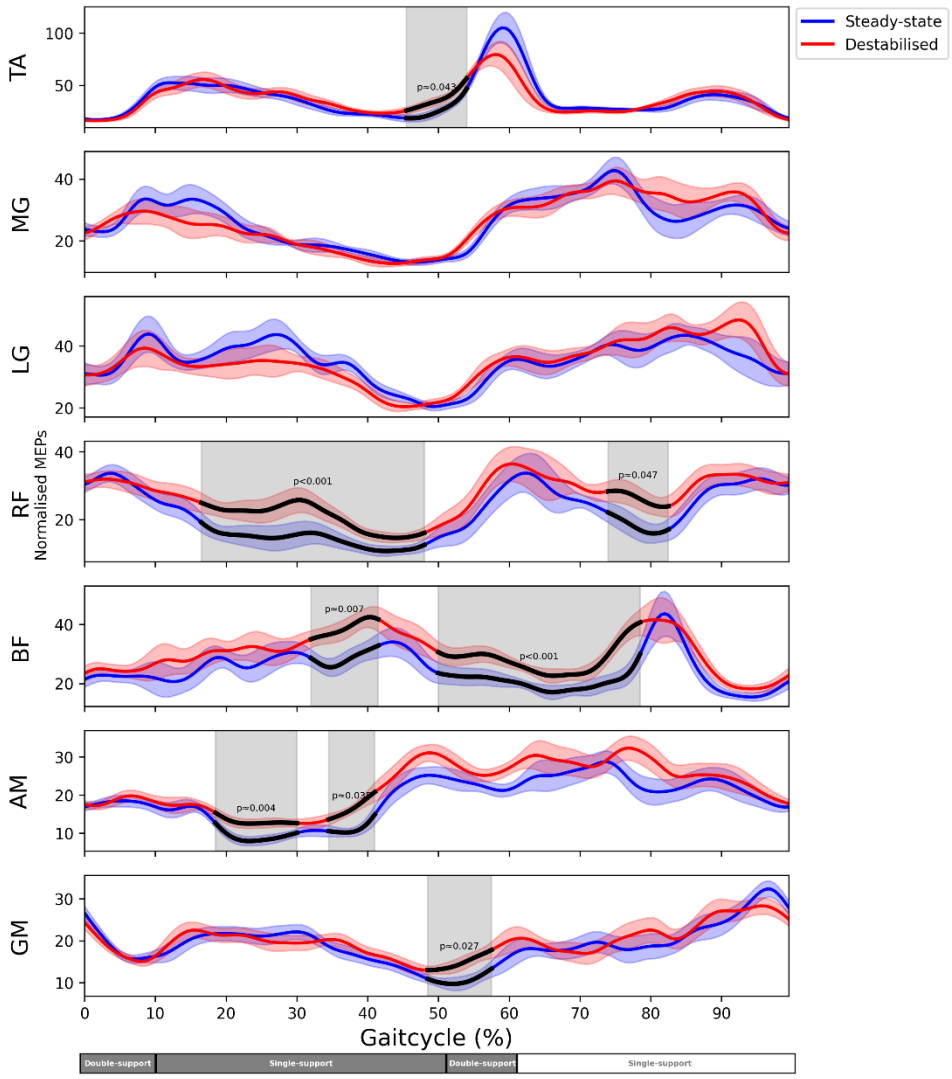

Figure 10. *Normalised MEPs are displayed over the gait cycle for all muscles. The plot starts at 0% of the gait cycle which corresponds to the right heel strike. The coloured shaded area's are the standard deviations of the conditions. The black lines and with the grey shade around it indicate significant difference. M. gastrocnemius medialis (MG), m. gastrocnemius lateralis (LG), m. rectus femoris (RF), m. biceps femoris (BF), m. adductor magnus (AM)*