Dynamics of corticospinal motor control during overground and treadmill walking in humans

Luisa Roeder[1], Tjeerd W Boonstra[2,3], Graham K Kerr[1]

[1] Institute of Health and Biomedical Innovation, School of Exercise and Nutrition Sciences, Queensland University of Technology, Brisbane, Australia

[2] Black Dog Institute, University of New South Wales, Sydney, Australia

[3] Systems Neuroscience Group, QIMR Berghofer Medical Research Institute, Brisbane, Australia

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Address for correspondence:

Luisa Roeder, Ph.D.

Institute of Health and Biomedical Innovation

School of Exercise and Nutrition Sciences

Queensland University of Technology

60 Musk Ave, Kelvin Grove, Queensland 4059, AUSTRALIA

Tel: +61 73138 6428; Fax: +61 73138 6030

Email: l.roeder@qut.edu.au

Abstract

Increasing evidence suggests cortical involvement in the control of human gait. However, the nature of corticospinal interactions remains poorly understood. We investigated time-frequency analysis of electrophysiological activity acquired during treadmill and overground walking in 22 healthy, young adults. Participants walked at their preferred speed (4.2, SD 0.4 km h^{-1}), which was matched across both gait conditions. Corticomuscular coherence (CMC) was assessed between EEG from bilateral sensorimotor cortices and EMG from the contralateral tibialis anterior (TA) muscle. Cortical power and CMC at theta, alpha, beta and gamma frequencies (4-45 Hz Hz) increased during the double support phase of the gait cycle for both overground and treadmill walking. High beta (21-30 Hz) CMC and theta (4-7 Hz) power was significantly increased during overground compared to treadmill walking. The phase spectra revealed positive time lags from EEG to EMG at alpha, beta and gamma frequencies, indicating that corticospinal interactions were governed by efferent activity. Phasic modulation of synchronization indicates that cortical control of human gait is not continuous but confined to the double support phase of the gait cycle. Frequency-band dependent differences in cortical and corticospinal synchronization between overground and treadmill walking suggest altered neural control for the two gait modalities, emphasizing the task-dependent nature of corticospinal processes during walking in humans.

New & Noteworthy

We investigated corticospinal dynamics during overground and treadmill walking in healthy adults. Corticospinal interactions at alpha, beta and gamma frequencies were of efferent nature and confined to the double support phase of the gait cycle. At high-beta frequencies corticospinal interactions were enhanced during overground compared to treadmill walking. These findings identify neurophysiological mechanisms that are important for understanding cortical control of human gait in health and disease.

Keywords: human gait, corticomuscular coherence, ambulatory EEG, neural oscillations, time-frequency analysis

Introduction

The control of human locomotion has commonly been thought to be driven by spinal and subcortical neural circuits. This assumption has been primarily based on animal studies, which have shown that cortical networks were only involved in generating locomotor activity in animals during more demanding walking tasks, such as precision stepping or obstacle avoidance (Armstrong 1988; Drew et al. 2008; Grillner 1985). However, increasing evidence from human neuroimaging studies suggests that cortical structures contribute to the control of simple, steady-state human gait (Fukuyama et al. 1997; Gwin et al. 2011; la Fougère et al. 2010; Miyai et al. 2001; Petersen et al. 2012; Seeber et al. 2014). However, the nature of corticospinal interactions remains poorly understood.

In recent years ambulatory electroencephalography (EEG), in conjunction with time-frequency analysis, has been increasingly used to investigate cortical and corticospinal sensorimotor processes during walking in humans (Artoni et al. 2017; Bradford et al. 2016; Bruijn et al. 2015; Bulea et al. 2015; Gwin et al. 2011; Knaepen et al. 2015; Oliveira et al. 2017b; Petersen et al. 2012; Seeber et al. 2014; Seeber et al. 2015; Severens et al. 2012; Sipp et al. 2013; Storzer et al. 2016; Wagner et al. 2016; Wagner et al. 2012; Wagner et al. 2014; Winslow et al. 2016). These studies have shown that cortical and corticospinal oscillations are modulated relative to the gait cycle at theta (4-7 Hz), alpha (8-12 Hz), beta (13-30 Hz) and gamma (>30 Hz) frequencies. However, there are considerable discrepancies in the precise temporal and frequency patterns of cortical dynamics, probably due to the wide variety of walking tasks used (e.g. treadmill walking at different speeds, on gradients, with additional gait-stability-

challenging tasks, with robotic assistance). For instance, beta oscillations were found to be enhanced during the double support phases of the gait cycle (event-related synchronization, ERS) and to be suppressed during the swing and single support phases (event-related desynchronization, ERD) (Artoni et al. 2017; Bradford et al. 2016; Bruijn et al. 2015; Bulea et al. 2015; Cheron et al. 2012; Gwin et al. 2011; Knaepen et al. 2015; Severens et al. 2012). By contrast, other groups observed ERS at 24-40 Hz (low gamma) during early and mid-swing, and ERD towards the end of the swing phase and during double support (Seeber et al. 2014; Seeber et al. 2015; Storzer et al. 2016; Wagner et al. 2012; Wagner et al. 2014).

There are also some initial reports which suggest that these cortical oscillations are transmitted to spinal motoneurons during walking in humans (Artoni et al. 2017; Petersen et al. 2012; Winslow et al. 2016). In nine participants, Petersen *et al.* (2012) showed corticomuscular coherence (CMC) between electroencephalographic (EEG) and electromyographic (EMG) signals from the tibialis anterior muscle (TA) at 8–12 and 24–40 Hz during walking. CMC was observed at approximately 400 ms prior to heel strike at two different walking speeds (1 km h^{-1} and 3.5–4 km h^{-1}). It is uncertain which exact phase of the gait cycle this would correspond to, as 400 ms before heel strike would coincide with different phases of the gait cycle at these two walking speeds (Castermans and Duvinage 2013). The authors suggested that CMC at 24-40 Hz during walking represents an efferent drive from cortex to spinal motoneurons, as they observed a negative imaginary part of coherency indicating a positive time lag from EEG to EMG (Petersen et al. 2012); however, the absolute time lag was not investigated. Recently, Artoni and colleagues (2017) reported a descending connectivity from motor cortex to leg muscles during undemanding, steady-state treadmill walking,

which was strongest for muscles of the swing leg but also present for muscles of the stance leg.

The vast majority of previous studies that investigated gait-related cortical oscillations, have been conducted on standard, motorized treadmills. Overground walking, however, has been shown to differ from treadmill walking dynamically and mechanically (Alton et al. 1998; Arsenault et al. 1986; Carpinella et al. 2010; Chiu et al. 2015; Dingwell et al. 2001; Lee and Hidler 2008; Ochoa et al. 2017; Riley et al. 2007; White et al. 1998). For example, treadmill walking has been found to reduce kinematic variability (Chiu et al. 2015; Dingwell et al. 2001), increase local dynamic stability (Dingwell et al. 2001), affect inter-limb coordination (Carpinella et al. 2010), modify lower limb muscle activation patterns, and joint moments and powers (Lee and Hidler 2008). These observations suggest that sensorimotor control of locomotion may vary between these two gait modalities, potentially affecting processes at the level of the cortex.

In the present study, we investigate sensorimotor cortical oscillations and corticospinal interactions during treadmill and overground walking in a large sample of healthy, young adults. By examining changes in cortical power and CMC during the gait cycle, we investigate the temporal and spectral profile of corticospinal interactions during human walking. The ‘constant phase shift plus constant time lag model’ (Mima and Hallett 1999) was used to estimate the time lag from the corresponding phase spectra and investigate the direction of corticospinal interactions. These findings elucidate the corticospinal dynamics involved in human gait.

Materials and Methods

Twenty-four healthy young adults (mean (SD), age 25.9 (3.2) years, height 170.4 (9.5) cm, weight 68.8 (12.1) kg; 12 men and 12 women) participated in the study. All experimental protocols were approved by the Human Research Ethics Committee of Queensland University of Technology (#1300000579) in accordance with the Declaration of Helsinki, and all participants gave written informed consent prior to participation.

Experimental protocol

All participants performed 12-14 minutes of overground walking and approximately seven minutes of treadmill walking at their preferred walking speed (3.3 – 4.8 km h^{-1}). The order of treadmill and overground walking was randomised across participants. The first two minutes of each trial served as familiarisation period and was not included in the analysis. For the overground condition, participants walked back and forth along a straight path (approximately 14 m) on a firm surface in the gait laboratory and turned at each end of the room. The turning sections, including acceleration at the beginning of the straight-line path and deceleration at the end (approximately 2.4 m), were labelled by the examiner and not included in further analyses (leaving 8.9 m of straight-line walking for the analysis). In order to obtain a similar amount of straight-line walking data in the overground condition, participants were required to walk for a longer duration (12-14 min) than in the treadmill condition (7 min). Treadmill walking was performed on a conventional treadmill (Nautilus, Pro Series). Both walking conditions were completed barefoot. Participants were instructed not to blink

excessively, to reduce swallows and to relax their face, jaw, and shoulder muscles during the data acquisition periods in order to minimise artefacts in the EEG recordings. Participants rested for five minutes between overground and treadmill walking in order for residual walking-specific after-motion-effects to dissipate and to prevent fatigue.

Gait velocity was matched for treadmill and overground walking on an individual participant basis. To this end, participants completed a 'pre-trial' (overground) of approximately three minutes before the actual experiment commenced in order to determine their preferred walking speed. Their mean walking velocity during the pre-trial was used to set the treadmill belt speed and to monitor and maintain the participants' walking speed consistent during the overground walking condition. If the participant did not comply with the pre-determined mean walking speed during the overground condition, they were instructed to walk faster/slower.

Data acquisition

Paired bipolar surface EMG (Ag-AgCl electrodes, 1 cm^2 recording area, 2 cm between poles) was recorded from the belly of the left and right TA. The EMG signals were filtered at 1-1000 Hz. Simultaneous EEG recordings were made using water-based AgCl electrodes placed on the scalp at 10 cortical sites according to the international 10-20 standard (P3, P4, C3, Cz, C4, F7, F3, Fz, F4, F8) using a 24-electrode cap (Headcap for water-based electrodes, TMSi, The Netherlands). Combined mastoids (A1, A2) were used as reference. The ground electrode was situated on the wrist using a wristband (TMSi, The Netherlands). EEG signals were filtered at 1-500 Hz. Footswitches were attached onto the participants' sole at the heel and the big toe of both feet. EEG, EMG, and footswitch signals were recorded with a wireless 32-channel amplifier system

(TMSi Mobita, The Netherlands) and sampled at 2 kHz. The recording system and wireless transmitter were placed in a belt bag and tied around the participants' waist.

Data analysis

All processing of data and spectral analyses were performed in MATLAB (2017a) using custom written routines.

Temporal gait parameters

Temporal gait parameters were extracted from the footswitch recordings. Time points of heel strike and toe-off for both feet were extracted and several gait parameters were calculated including stride time, step time, stride- and step time variability, cadence, swing phase, stance phase, single- and double-support time. Walking velocity was calculated as m s^{-1} from the time required to complete the 8.9 m of straight-line walking during overground walking.

Data pre-processing and artefact removal

Prior to spectral analysis, electrophysiological data were pre-processed and artefacts were removed. EEG channels were visually inspected and segments with excessive noise (large-amplitude movement artefacts, EMG activity) were rejected. Next, EEG signals were band-pass filtered (2nd order Butterworth, 0.5-70 Hz) and re-referenced to a common average reference (re-referencing was performed in EEGLAB version 13.6.5, Delorme and Makeig 2004). A common average reference in the post-processing steps has been shown to minimize motion artefacts in EEG signals (Snyder et al. 2015). Overground walking EEG recordings were truncated to the straight-line walking segments (turning segments were removed). Subsequently, independent

component (IC) analysis was performed on the concatenated segments using the infomax ICA algorithm implemented in EEGLAB. ICs containing eye blink, muscle, or movement artefacts were removed from the data (on average 3.7 ICs were removed per participant); the remaining ICs were retained and projected back onto the channels.

A bipolar EEG montage was used to assess cortical activity from bilateral motor cortices: C3-F3 for the left motor cortex, and C4-F4 for the right motor cortex (cf. Long et al. 2015). A differential recording between the electrode pairs is most sensitive to activity locally generated between the electrode pairs and suppresses more distant activity. Hence, it can be used to minimize artefacts similar to a common reference approach (e.g. Petersen et al. 2012; Snyder et al. 2015).

EMG data were high-pass filtered (4th order Butterworth, 10 Hz cut-off) and full-wave rectified using the Hilbert transform. There is some discussion on the use of EMG rectification for the estimation of CMC (Halliday and Farmer 2010; McClelland et al. 2012; Neto and Christou 2010). For assessing CMC at low force levels, it has been shown that rectification is appropriate (Boonstra and Breakspear 2012; Farina et al. 2013; Ward et al. 2013). We therefore rectified the EMG signals, as walking qualifies as low force movement. Moreover, rectification of EMG signals in the process of calculating CMC has been applied in previous ambulatory CMC studies (Petersen et al. 2012).

After rectification, EMG signals were demodulated to avoid spurious coherence estimates resulting from periodic changes in EMG amplitude (Boonstra et al. 2009). Walking periodically activates the muscles and results in a rapid increase in EMG amplitude during each gait cycle. These fluctuations in EMG amplitude were removed by demodulating the EMG signal using the Hilbert transform to obtain the instantaneous

phase $\theta(n)$ of the rectified EMG signal. The demodulated EMG signal $y(n)$ can be defined as the cosine of the instantaneous frequency or phase:

$$y(n) = \cos\{\theta(n)\}$$

where $y(n)$ has amplitude 1 and the same instantaneous phase as the original rectified EMG signal (Boonstra et al. 2009).

Spectral analysis

Time-frequency coherence was estimated pairwise between bipolar EEG signals (C4-F4, C3-F3) and rectified and demodulated EMG signals of the contralateral TA muscle (TAl, TAr) with respect to heel strike of the left and right foot to assess changes in corticospinal synchronization during the gait cycle of overground and treadmill walking. To this end, EEG and EMG signals were segmented into L = 220 segments of length T = 1000 ms (-800 to +200 ms with respect to heel strike of the right or left foot) for each participant and condition. Heel strike served as reference point (t = 0) in the gait cycle to which all segments were aligned. Event-related EEG power spectra and coherence between EEG and EMG (Mehrkanoon et al. 2014) were computed across the 220 segments using a 375-ms Hanning window and a sliding window method with increments of 25 ms. The window length was determined after visual inspection of different window settings with simulated data and experimental data of a representative participant, showing that a window of 375 ms optimised the trade-off between spectral and temporal resolution.

EEG power spectra were expressed as percentage change from the average by subtracting and dividing by the mean across the time interval separately for each frequency (Boonstra et al. 2007). Power and coherence spectra were computed for each

participant during overground and treadmill walking, and subsequently averaged across participants to render the grand-average for each estimate (C3F3-TAr, C4F4-TAl) and condition (overground, treadmill). Additionally, event-related EMG envelopes were computed using filtered and rectified EMG data.

To investigate the timing relationship between EEG and EMG activity, we assessed the phase difference between both signals (Halliday et al. 1995). The time lag and phase offset between two signals can be determined from the phase spectra. That is, a constant time lag between two signals results in a linear trend in the phase difference across frequencies (Mima and Hallett 1999). To estimate the time lag from the phase spectra, we fitted a line to the phase spectrum across the frequency range at which significant coherence was observed, and the time lag can be directly calculated from the slope of the regression line (Mehrkanoon et al. 2014; Raethjen et al. 2002). Time lags were separately calculated for different frequency bands and conditions. To this end, coherency was averaged across C3F3-TAr and C4F4-TAl to improve the signal-to-noise ratio. Subsequently, coherence and phase were extracted from these averaged coherency estimates, and time lags were calculated for theta, alpha, low beta, high beta and gamma frequency ranges.

Statistical analysis

Paired sample t-tests (SPSS, version 23) were used to compare gait parameters across conditions (overground, treadmill).

To assess whether the coherence estimates were statistically significant in individual participants, confidence intervals (CI) were constructed based on the number

of segments included in the analysis. The 95% confidence limit was estimated as (Amjad et al. 1997)

$$CI = 1 - (alpha)^{1/(L-1)} \quad (1)$$

A two-stage summary statistics approach was used to assess statistical significance of time–frequency coherence at group level. First, magnitude-squared coherence of individual participants was transformed to z-scores using a parametric approach (Halliday et al. 1995). To this end, we computed the p-value and converted it to a z-score using the standard (normal) distribution. Rewriting Eq. (1), the p-value of coherence can be estimated as

$$p = 1 - (1 - C)^{(L-1)}, \quad (2)$$

where C is magnitude-squared coherence. Using the normal distribution the p-value can be converted to a z-score, which has an expected value of 0 and a standard deviation of 1 under the null hypothesis (EEG and EMG signals are independent). Subsequently, a one-sample t-test was used to test whether the individual z-scores were significantly different from zero (Mehrkanoon et al. 2014).

Linear mixed models (LMM) were used to compare the repeated measures of z-transformed coherence across conditions (overground, treadmill). Mean z-transformed coherence over salient time-frequency windows was used for the analysis. Five time-frequency windows were identified: Double support (0 to 125 ms at/after heel strike) at (i) theta (4-7 Hz), (ii) alpha (8-12 Hz), (iii) lower beta (13-20 Hz), (iv) higher beta (21-30 Hz), and (v) gamma frequencies (31-45 Hz). The fixed effect factor included in the model was condition. A random effect for participant (i.e. using the participant identification number) was included, which takes into account the repeated coherence

measures among participants. Statistically significant main effects were followed up with post-hoc comparisons using a Bonferroni adjustment for multiple comparisons. The LMM analysis was performed in SPSS (version 23). The same model was used to compare EEG power across conditions (overground, treadmill). Mean normalised PSD over the salient time-frequency windows per participant was used for the analysis.

An intercept-LMM with a fixed effect factor for condition was used to compare the time lags of the different frequency bands against zero, and across overground and treadmill walking. A significant intercept represents a rejection of the null hypothesis and indicates a non-zero time lag between EEG and EMG signals at group level. A significant effect for condition indicates a difference in time lag between overground and treadmill walking.

Alpha was set at 0.05 for all analyses.

Results

Two participants' datasets were excluded from the spectral analysis (i.e. for all spectral outcome measures: power, coherence and phase) due to excessive EEG artefacts across all channels and conditions (large-amplitude movement artefacts). Another two participants' datasets during treadmill walking were excluded, because fewer than 100 left heel strike triggers were extracted. Thus, group mean estimates for spectral analyses relative to heel strike of the left foot during treadmill walking (i.e. C4F4 for power, and C4F4-TAl for coherence and phase) were based on n = 20 participants. All other spectral estimates (C3F3-TAr coherence/phase and C3F3 power

overground and treadmill, C4F4-TAl coherence/phase and C4F4 overground) were based on n = 22 participants.

Temporal gait parameters

Participants were instructed to walk at their preferred speed, which was matched across both gait conditions. Mean walking speed was not significantly different between overground and treadmill walking ($p = 0.362$). However, walking speed values were missing for three participants; hence, the group mean estimate was based on n = 19 participants. Table 1 presents all temporal gait parameters during overground and treadmill walking extracted from the footswitch recordings.

Most temporal gait parameters were not significantly different between overground and treadmill walking: including duration of step time, step- and stride time variability, swing, single and double support phases. However, stride time was significantly longer during overground than during treadmill walking ($p = 0.007$), and there was a trend towards an extended stance phase during overground walking ($p = 0.054$). Moreover, participants walked at a higher cadence on the treadmill than overground ($p = 0.012$).

EMG envelope

Figure 1A and B shows the event-related EMG envelopes for the left and right TA during overground and treadmill walking. The pattern shows increased amplitude during early swing (around -300 ms prior to heel strike), as well as during late swing and early stance (-100 ms prior to +100ms after heel strike). During mid swing (around -200 ms prior to heel strike) the amplitude decreased. The envelope is marked by a

double peak at the time point of heel strike (t = 0) when the foot is fully dorsiflexed and at 60 ms after heel strike (early double support). The amplitude increase during early swing already commenced before toe-off of the swing leg.

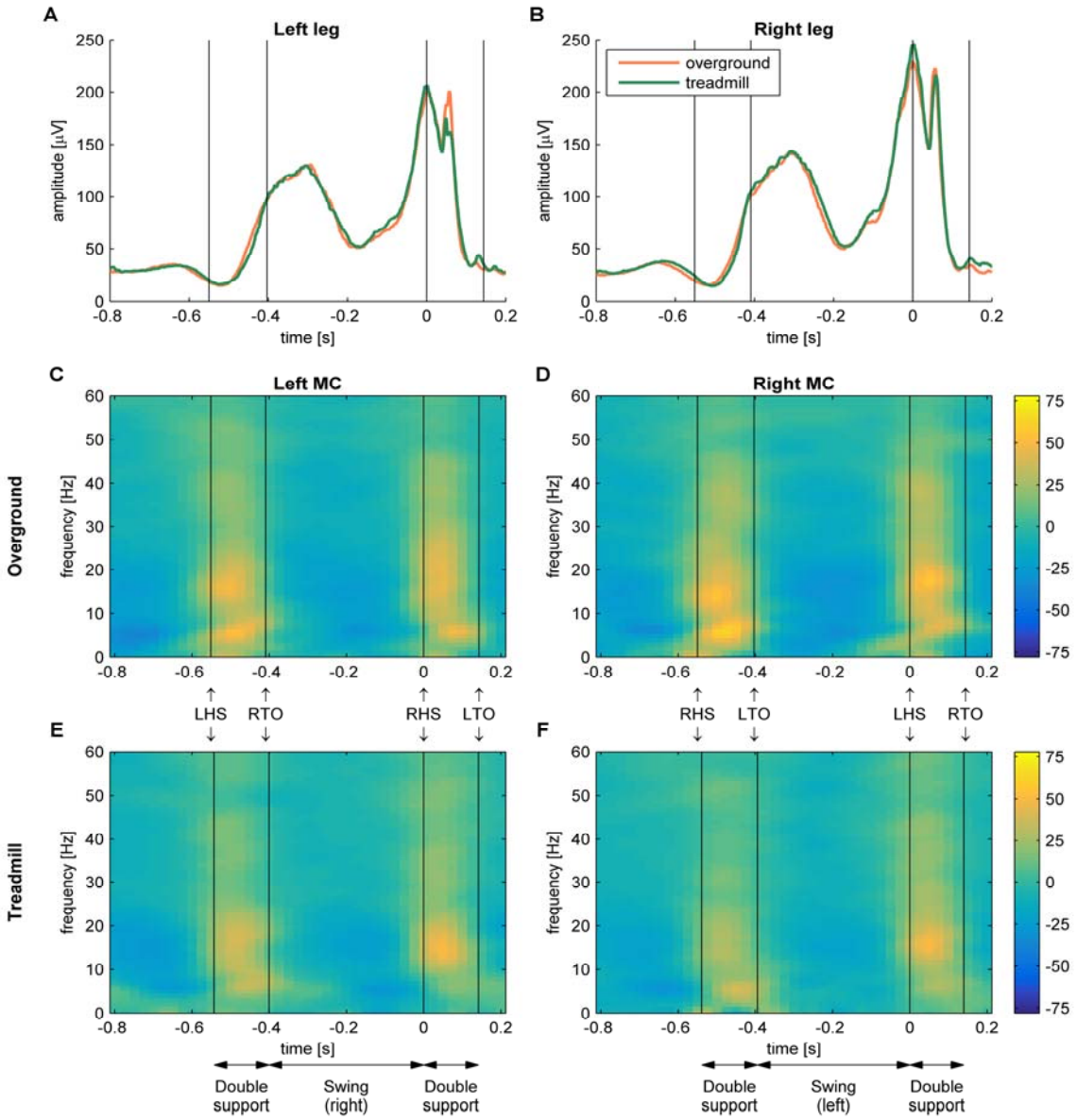

Figure 1. Grand-average EMG envelopes and time-frequency EEG power spectra.

EMG envelopes of the left (A) and right (B) TA during overground (orange) and treadmill (green) walking. EEG PSD acquired from bipolar EEG signals of the left motor cortex (C3-F3) during overground (C) and treadmill walking (E), and of the right motor cortex (C4-F4) during overground (D)

and treadmill walking (F), showing the percent change from the average. X-axis shows time points of gait cycle in seconds, relative to heel strike (t=0) of the left (B, D, F) and right (A, C, E) foot. LHS, left heel strike; LTO, left toe-off; MC, motor cortex; RHS, right heel strike; RTO, right toe-off; TA, tibialis anterior.

Event-related synchronization

The amplitude modulations of cortical activity revealed a different pattern. Increased EEG power (ERS) was observed across a range of frequencies during double support, while power reduced (ERD) during the swing and single support phases (Figure 1C to F). The greatest power increase was observed during double support between approximately 15 to 25 Hz, with an increase of up to 55% relative to the average across the whole gait cycle.

The statistical comparison using LMM revealed that ERS during double support in the theta band was significantly greater during overground compared to treadmill walking ($F(1, 63.90) = 5.04$, $p = 0.03$; Figure 2). The increase in theta power was 29.7% during double support for overground and 15.2% for treadmill walking; the estimated mean difference was 14.5% [95% CI 1.6, 27.4]. No significant differences ($p \geq 0.14$) between overground and treadmill walking were observed for the other frequency bands (Figure 2).

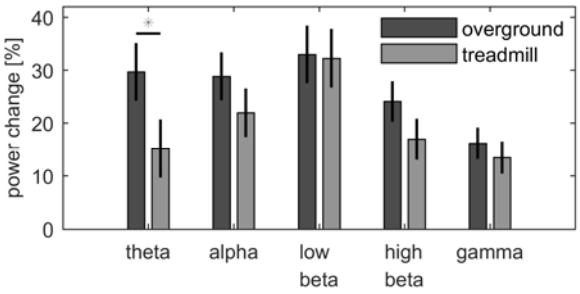

Figure 2. Grand-average EEG power during double support.

Normalized EEG PSD during double support (0-125 ms) averaged across sides/channels (C3F3, C4F4), depicted for each frequency band (theta, alpha, low beta, high beta, gamma) during overground and treadmill walking. Bars show the group mean; error bars depict the standard error of the mean. Theta PSD was significantly greater during overground compared to treadmill walking.

Corticomuscular coherence

During both gait conditions CMC increased during the double support phases of the gait cycle at frequencies between 0 to approximately 45 Hz and little or no CMC was observed during the swing and single support phases (Figure 3). During the double support phase, CMC decreased at higher frequencies showing the highest CMC for frequencies <10 Hz, medium CMC for frequencies 11-20 Hz, lowest CMC for frequencies >25 Hz. CMC was statistically significant during double support in all frequency bands for both sides/channels and gait conditions ($p < 0.02$).

The statistical comparison of the gait conditions using LMMs revealed that CMC in the high beta band was significantly greater during overground compared to treadmill walking ($F(1, 63.69) = 4.44$, $p = 0.04$; Figure 4). Grand-average z-transformed high beta coherence was 1.90 during overground and 1.31 during treadmill walking; the estimated mean difference was 0.59 [95% CI 0.03, 1.14]. No significant differences between

overground and treadmill walking were observed in the other frequency bands ($p \geq 0.06$).

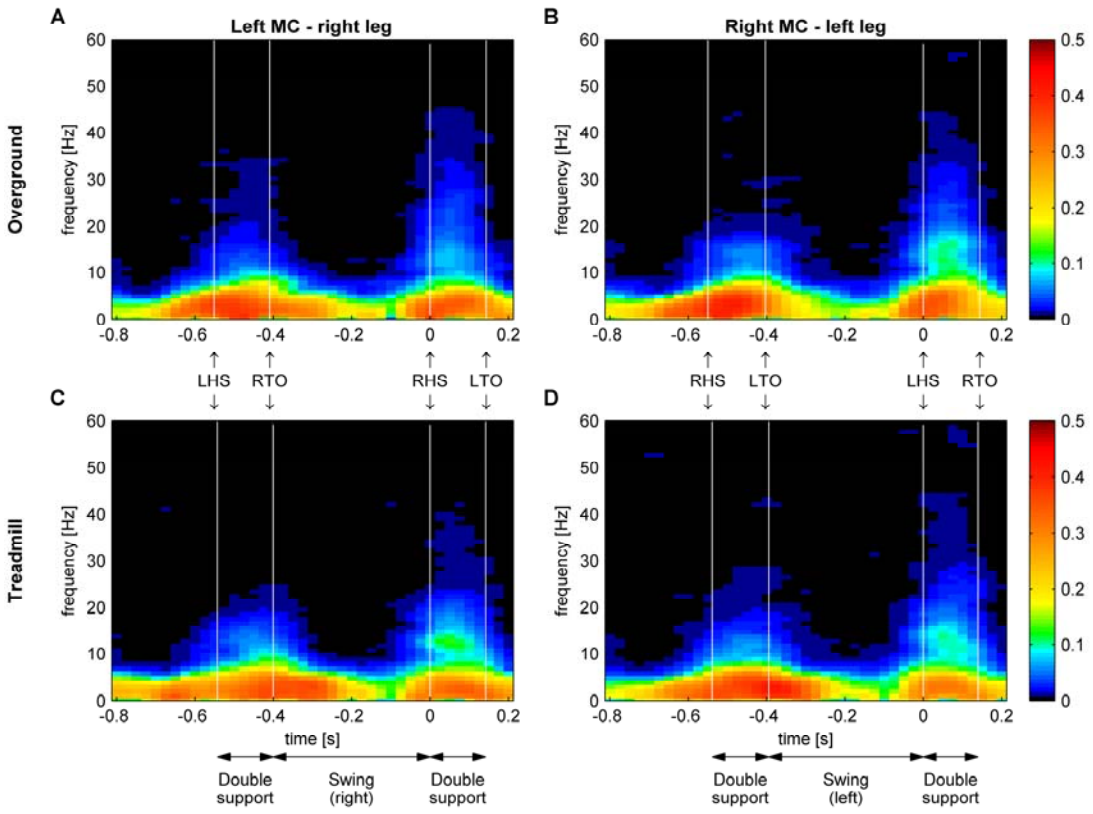

Figure 3. Grand-average time-frequency coherence between EEG and EMG.

Corticomuscular coherence between bipolar EEG from the left motor cortex (C3F3) and EMG from the right TA during overground (A) and treadmill (C) walking; coherence of bipolar EEG from the right motor cortex (C4F4) and EMG from the left TA during overground (B) and treadmill (D) walking. X-axis shows time points of gait cycle in seconds, relative to heel strike (t=0) of the left (B, D) and right (A, C) foot. Y-axis shows frequencies in Hz. Colour-coding shows the coherence value. LHS, left heel strike; LTO, left toe-off; MC, motor cortex; RHS, right heel strike; RTO, right toe-off; TA, tibialis anterior.

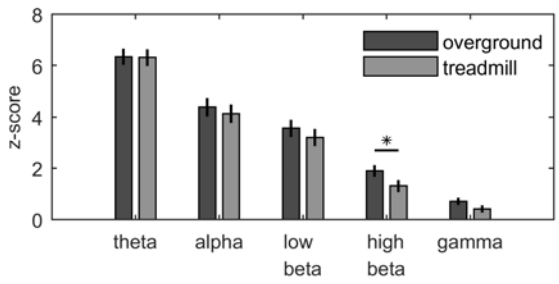

Figure 4. Grand-average coherence during double support.

Z-transformed coherence during double support (0-125 ms) averaged across sides/channels (C3F3-TAr, C4F4-TAl), depicted for each frequency band (theta, alpha, low beta, high beta, gamma) during overground and treadmill walking. Bars show the group mean; error bars depict the standard error of the mean. High beta z-transformed coherence was significantly greater during overground compared to treadmill walking.

Phase spectra

The time delay between EEG and EMG was estimated during overground and treadmill walking separately in each frequency band (theta, alpha, lower beta, higher beta, gamma; Figure 5). We extracted the coherence spectrum in the middle of the double support phase (t = 50 ms) and determined for each participant the frequency bins at which CMC was significant. A linear line was fitted to these frequency bins of the corresponding phase spectra and the time lag was estimated from the slope of the fitted line (see Figure 5A and B for an example in the lower beta band).

Statistical analyses of time lag revealed that time lag during double support was greater than zero at in the alpha ($F(1, 33) = 13.33$, $p = 0.0009$), low beta ($F(1, 39) = 6.88$, $p = 0.0124$), high beta ($F(1, 25) = 19.44$, $p = 0.0002$), and gamma bands ($F(1, 17) = 7.84$, $p = 0.0123$), but not in the theta band ($p = 0.84$). This indicates that EEG signals

were leading EMG signals in these frequency bands. The grand-average time lag was 30.0 ms [95% CI 13.3, 46.8] in the alpha band, 11.4 ms [95% CI 2.6, 20.8] in low beta, 26.5 ms [95% CI 14.1, 38.9] in high beta, 18.3 ms [95% CI 4.5, 32.0] in the gamma band (Figure 5C). No significant differences in time lag were observed between overground and treadmill walking ($p \geq 0.15$).

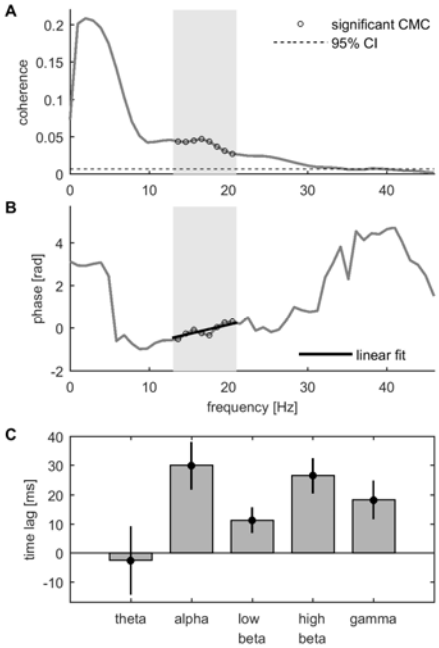

Figure 5. Estimation of time lag between EEG and EMG.

(A) Corticomuscular coherence between bipolar EEG from the motor cortex and contralateral TA EMG at the time point +50 ms (mid double support) during overground walking. Data were averaged across sides/channels (C3F3-TAr, C4F4-TAl). Grey patches indicate low beta frequency range (13-21 Hz), dots depict FFT frequency bins with significant CMC, horizontal dashed line shows upper limit of 95% CI. (B) Corresponding phase spectrum and fitted regression line in the lower beta band. (C) Estimated mean time lag between EEG and EMG in each frequency band (averaged across overground and treadmill, as statistical analyses identified no significant effect of gait condition on time lag). Bars and dots show the group mean; vertical lines depict the standard error of the mean. Time lags estimated based on slope of fitted regression line of individual phase spectra in each frequency band and condition. Positive time lag values indicate a time lag from EEG to EMG. CI, confidence interval; CMC, corticomuscular coherence.

Discussion

CMC and power were examined during overground and treadmill walking to investigate corticospinal dynamics during both. Significant CMC at theta, alpha, beta and gamma frequencies (4-45 Hz) was found between motor cortex and the contralateral TA muscle during the double support phase. During the swing phase CMC was largely absent. Similarly, EEG power at these frequency bands increased during double support and decreased during swing for both gait conditions. High beta CMC and theta EEG power was significantly enhanced during overground compared to treadmill walking. Alpha, beta and gamma CMC during double support showed a positive time lag between EEG and EMG, suggesting that cortical activity was leading muscle activity. Most temporal gait parameters were not significantly different between overground and treadmill walking, except stride time (significantly longer overground) and cadence (significantly lower overground).

Increased synchronization during double support

For both overground and treadmill walking, we found that CMC and EEG power increased during the double support phase of the gait cycle and decreased during swing/single support. Cortical and corticospinal synchronization increased at a broad frequency range, including theta, alpha, beta and gamma frequencies. These results highlight that cortical and corticospinal oscillations are modulated dependent on the

phase of the gait cycle and suggest that the cortex is intermittently involved in controlling human gait (rather than a continuous drive).

The cortical power fluctuations during overground and treadmill walking observed in this study closely resemble those previously reported in ambulatory EEG studies conducted on a treadmill. A number of studies found ERS over sensorimotor cortical areas at the end of stance and during double support, and ERD during the swing/single support phases (Artoni et al. 2017; Bradford et al. 2016; Bruijn et al. 2015; Bulea et al. 2015; Cheron et al. 2012; Gwin et al. 2011; Knaepen et al. 2015; Oliveira et al. 2017b; Severens et al. 2012). Notably, one previous study (Bulea et al. 2015) reported similar changes in cortical power to those in the current study during walking on a user-driven treadmill, a condition which simulates overground walking more closely than walking on a standard, motorized treadmill. However, other studies reported ERS during the swing phase and ERD during double support (Seeber et al. 2014; Seeber et al. 2015; Storzer et al. 2016; Wagner et al. 2012; Wagner et al. 2014). Walking tasks in these studies included treadmill walking in a robotic driven gait orthosis (Seeber et al. 2014; Seeber et al. 2015; Wagner et al. 2012; Wagner et al. 2014) and overground walking (Storzer et al. 2016). While robot assisted walking is quite different from steady-state treadmill walking, which may help to explain discrepant findings, the changes in cortical power found during overground walking by Storzer *et al.* (2016) differ substantially from our results. In their study participants were required to walk at a cadence of 40 strides per minute (although no audio-visual cues were provided to ensure compliance) and walking speed was not kept constant throughout the trial, whereas our participants chose their own cadence for each gait condition (approximately 55 strides per minute) and walking speed was kept constant (~4 km h^{-1}).

The time-frequency profiles of CMC largely match the profiles of cortical oscillations. We found increased ERS and CMC during double support suggesting that increased oscillatory synchrony in sensorimotor cortical areas was also transmitted to the spinal cord. This is supported by the positive time lags at alpha, beta and gamma frequencies between EEG and EMG activity, which indicated that corticospinal interactions were dominated by efferent activity. Thus, during double support cortical neurons may tend to synchronize and influence the discharge behavior of the spinal motoneurons that innervate leg muscles. To our knowledge, only one study (Petersen et al. 2012) and one case-report (Winslow et al. 2016) have previously investigated CMC during walking. Petersen et al. (2012) found significant CMC between motor cortex and TA at 8-12 and 24-40 Hz during treadmill walking, which partly overlap with the frequency ranges in the current study. While both studies show corticospinal interactions during steady-state walking in humans, the temporal profiles of CMC may differ: We found CMC mainly during double support, whereas Petersen et al. (2012) observed CMC ~400 ms prior to heel strike (possibly corresponding to the swing phase). Further experimental investigation of CMC between cortex and leg muscles other than the TA during walking would help to resolve the timing of intra-stride modulations of CMC, as one would expect to find CMC at different phases of the gait cycle. However, it is worthwhile noting that during dynamic ankle movements beta CMC has been observed during dorsiflexion for two antagonistic muscles (Yoshida et al. 2017). Artoni and colleagues (2017) recently showed a descending connectivity from motor cortical regions to different leg muscles during the swing phase of steady-state treadmill walking. This unidirectional, top-down connectivity was strongest for muscles of the swing leg but also present for muscles of the stance leg during the swing phase.

This connectivity was stronger for the TA muscles (of both swing and stance leg) than for other more proximal muscles. However, cortical-muscle network connectivity was only assessed during the swing phase but not during other phases of the gait cycle (e.g. stance or double support).

Studies that have investigated intramuscular coherence between populations of TA motoneurons (Barthelemy et al. 2010; Halliday et al. 2003; Hansen et al. 2005; Nielsen et al. 2008; Petersen et al. 2013; Petersen et al. 2010) also found significant coherence at frequencies below 50 Hz during treadmill walking. While intramuscular coherence does not necessarily reflect corticospinal drive, the frequency range of these results is in line with the present findings on CMC during treadmill and overground walking. Nielsen and colleagues (Halliday et al. 2003; Nielsen et al. 2008) have shown that intramuscular coherence is modulated with the gait cycle. They found that coherence was present during early swing, mostly absent during mid swing, and greatest during terminal swing and early double support. This is largely in agreement with the temporal profiles of CMC and the EMG envelopes found in the present study.

Frequency-specific differences between conditions

We found frequency-specific differences in ERS and CMC between overground and treadmill walking. ERS during double support was significantly increased at theta frequencies during overground compared to treadmill walking. Similarly, CMC at high beta frequencies (21-30 Hz) was significantly enhanced during overground walking. In line with previous research, this highlights that cortical oscillations are sensitive towards the walking task that is performed (Bradford et al. 2016; Bruijn et al. 2015; Bulea et al. 2015; Kline et al. 2016; Knaepen et al. 2015; Lisi and Morimoto 2015;

Oliveira et al. 2017b; Sipp et al. 2013; Storzer et al. 2016; Wagner et al. 2016; Wagner et al. 2012; Wagner et al. 2014). Cortical theta oscillations were modulated by the complexity of the locomotor task and by the phase of the gait cycle (i.e. transitions between gait cycle phases, Bradford et al. 2016; Bulea et al. 2015; Oliveira et al. 2017b; Sipp et al. 2013; Wagner et al. 2012). For instance, theta ERS in motor cortical regions has been found to be increased during more complex walking tasks, such as walking on a balance beam (Sipp et al. 2013), on a gradient (Bradford et al. 2016), or on an active, user-driven treadmill (Bulea et al. 2015). In the context of the present study, this would suggest that overground walking may be more demanding/challenging than walking on a motorized, standard treadmill, and hence be accompanied by increased theta ERS. During overground walking participants have to actively track their walking speed and temporospatial gait patterns themselves rather than simply following the treadmill belt speed, which dictates walking speed and influences temporospatial parameters externally. This would indeed be supported by our finding that treadmill walking decreased stride time and increased cadence. Theta ERS has also be linked to critical time points of the gait cycle, such as transitions between stance and swing, as a control strategy of postural stability (Bradford et al. 2016; Sipp et al. 2013). In that sense, the differences in theta ERS we found between overground and treadmill walking may be related to a difference in timing during overground walking.

High beta CMC was significantly enhanced during overground walking, and was identified as descending drive from cortex to muscle. To our knowledge, no previous study has compared corticospinal dynamics between locomotor tasks, but only investigated it during treadmill walking (Artoni et al. 2017; Petersen et al. 2012; Winslow et al. 2016). However, a number of studies have assessed cortical oscillations

during various gait tasks and have found task-dependent differences in cortical beta power (Bruijn et al. 2015; Bulea et al. 2015; Knaepen et al. 2015; Lisi and Morimoto 2015; Oliveira et al. 2017b; Sipp et al. 2013; Wagner et al. 2016; Wagner et al. 2012; Wagner et al. 2014). These studies have suggested that cortical beta oscillations may be related to controlling gait stability (Bruijn et al. 2015; Sipp et al. 2013), gait adaptations (i.e. step lengthening and shortening, Wagner et al. 2016), sensory processing of the lower limbs (Wagner et al. 2012), visuomotor integration (Oliveira et al. 2017b; Wagner et al. 2014), and speed control (Bulea et al. 2015; Lisi and Morimoto 2015). In our experiment, participants were required to perform steady-state overground and treadmill walking at the same speed and without any perturbations that would have triggered deliberate stepping adaptations. Hence, different levels of high beta CMC during overground and treadmill walking may reflect differences in gait stability, step adaptations, sensorimotor processing and/or speed control in each gait condition in our study. Possibly, corticospinal beta activity may be related to motor control mechanisms of stride time or cadence, as these parameters were significantly different between treadmill and overground walking in the present study. This interpretation is supported by studies that showed biomechanical differences between overground and treadmill gait, which are thought to be caused by neuromuscular control mechanisms (Carpinella et al. 2010; Chiu et al. 2015; Dingwell et al. 2001; Lee and Hidler 2008; Ochoa et al. 2017). Our study highlights that findings on cortical control of human gait inferred from studies conducted on a treadmill should be interpreted with caution, as sensorimotor processes during overground and treadmill walking may differ.

Efferent drive from cortex to muscle

We found positive time lags between coherent EEG and EMG activity at alpha, beta and gamma frequencies, which provides further evidence for efferent cortical control during walking in humans (Artoni et al. 2017; Petersen et al. 2012). We found similar time lags for overground and treadmill walking, suggesting that the same efferent pathways are involved in both locomotor tasks.

Time lags varied from approximately 12 ms in the lower beta band to 30 ms in the alpha band. This variance likely reflects the signal-to-noise ratio of estimating the slope across a limited number of data points. The time lag at low beta frequencies (~12 ms) is consistent with previous studies investigating beta-band CMC in upper limb muscles during isometric contractions, e.g. 11 ms (Mehrkanoon et al. 2014), 15.9 ms (Mima et al. 2000), 9.3 ms (Gerloff et al. 2006) and 7.9 ms (Witham et al. 2011). However, this time lag is shorter than the corticospinal conduction time from the cortex to the TA measured by stimulation (roughly 27 ms, Gross et al. 2000; Petersen et al. 2001; Rothwell et al. 1991; Schubert et al. 1997). This could be due to the state of the muscle (active during walking) or the calculation of the time delays based on phase difference. The time delay estimated from phase spectra also appears to underestimate the actual physiological corticospinal conduction times (Witham et al. 2011). Hence, while the absolute time lag found in the current study may be an underestimation of the actual conduction time, a positive time lag was very robust at the group level, indicating a cortical drive to the TA muscle during overground and treadmill walking.

In the theta band the time lag between coherent EEG and EMG activity was not significantly different from zero. This suggests that theta CMC may be related to a

different corticospinal process. However, the slope of the theta phase spectrum was estimated across only three frequency bins and should hence be interpreted with caution.

Limitations

Ambulatory EEG is susceptible to movement artefacts and caution should hence be exerted when interpreting phasic changes in EEG activity (Castermans et al. 2014; Costa et al. 2016; Gwin et al. 2010; Kline et al. 2015; Nathan and Contreras-Vidal 2016; Oliveira et al. 2016; Snyder et al. 2015). In this study, we applied multiple strategies to mitigate the effects of motion artefacts: We excluded gait cycles with excessive artefacts, we performed ICA, and we calculated a bipolar derivative EEG signal, which removes further shared artefactual activity across channels similar to a re-referencing approach (Snyder et al. 2015). This way, a bipolar EEG signal reflects nearby cortical activity that is locally generated between the electrode pairs. With respect to the EMG recordings, we high-pass filtered, rectified, and demodulated the signals in order to remove low-frequency artefacts and periodic amplitude modulations that could distort the coherence estimates. The positive time lags in the alpha, beta and gamma bands suggest that the CMC results of this study were not due to conduction of non-physiological artefacts, which would have likely resulted in a zero time lag (Petersen et al. 2012), but reflect genuine neural interactions between cortex to muscle. Oscillatory activity in the theta band revealed a different pattern, which may suggest residual artefact contamination at theta frequencies. Previous studies indeed found that oscillatory activity at low frequencies may be more affected by movement artefacts than at other/higher frequency ranges (Castermans et al. 2014).

The ERD-ERS profiles observed in this study are similar to those in multiple previous studies on cortical oscillatory activity during walking (Artoni et al. 2017; Bradford et al. 2016; Bruijn et al. 2015; Bulea et al. 2015; Cheron et al. 2012; Gwin et al. 2011; Knaepen et al. 2015; Severens et al. 2012). This provides further evidence that the observed time-frequency modulations of cortical power and CMC in the current study reflect genuine neural interactions. However, EEG acquisition during dynamic movement remains challenging and methods to minimize movement artefacts in ambulatory EEG is an ongoing pursuit (Kline et al. 2015; Oliveira et al. 2017a; Snyder et al. 2015).

Conclusion

This study shows that motor cortical and corticospinal synchrony are enhanced during the double support phase of the gait cycle during steady-state overground and treadmill walking in healthy, young adults. Corticospinal interactions at alpha, beta and gamma frequencies were identified as efferent mechanisms. Moreover, this study shows that corticospinal high-beta interactions and cortical theta oscillations differ between overground and treadmill walking, indicating altered motor control dependent on the gait modality. These findings identify neurophysiological mechanisms that are important for understanding cortical control of human gait in health and disease.

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Figure Captions

Figure 1. Grand-average EMG envelopes and time-frequency EEG power spectra.

EMG envelopes of the left (A) and right (B) TA during overground (orange) and treadmill (green) walking. EEG PSD acquired from bipolar EEG signals of the left motor cortex (C3-F3) during overground (C) and treadmill walking (E), and of the right motor cortex (C4-F4) during overground (D) and treadmill walking (F), showing the percent change from the average. X-axis shows time points of gait cycle in seconds, relative to heel strike (t=0) of the left (B, D, F) and right (A, C, E) foot. LHS, left heel strike; LTO, left toe-off; MC, motor cortex; RHS, right heel strike; RTO, right toe-off; TA, tibialis anterior.

Figure 2. Grand-average EEG power during double support.

Normalized EEG PSD during double support (0-125 ms) averaged across sides/channels (C3F3, C4F4), depicted for each frequency band (theta, alpha, low beta, high beta, gamma) during overground and treadmill walking. Bars show the group mean; error bars depict the standard error of the mean. Theta PSD was significantly greater during overground compared to treadmill walking.

Figure 3. Grand-average time-frequency coherence between EEG and EMG.

Corticomuscular coherence between bipolar EEG from the left motor cortex (C3F3) and EMG from the right TA during overground (A) and treadmill (C) walking; coherence of bipolar EEG from the right motor cortex (C4F4) and EMG from the left TA during overground (B) and treadmill (D) walking. X-axis shows time points of gait cycle in seconds, relative to heel strike (t=0) of the left (B, D) and right (A, C) foot. Y-axis shows frequencies in Hz. Colour-coding shows the coherence value. LHS, left heel strike; LTO, left toe-off; MC, motor cortex; RHS, right heel strike; RTO, right toe-off; TA, tibialis anterior.

Figure 4. Grand-average coherence during double support.

Z-transformed coherence during double support (0-125 ms) averaged across sides/channels (C3F3-TAr, C4F4-TAl), depicted for each frequency band (theta, alpha, low beta, high beta, gamma) during overground and treadmill walking. Bars show the group mean; error bars depict the standard error of the mean. High beta z-transformed coherence was significantly greater during overground compared to treadmill walking.

Figure 5. Estimation of time lag between EEG and EMG.

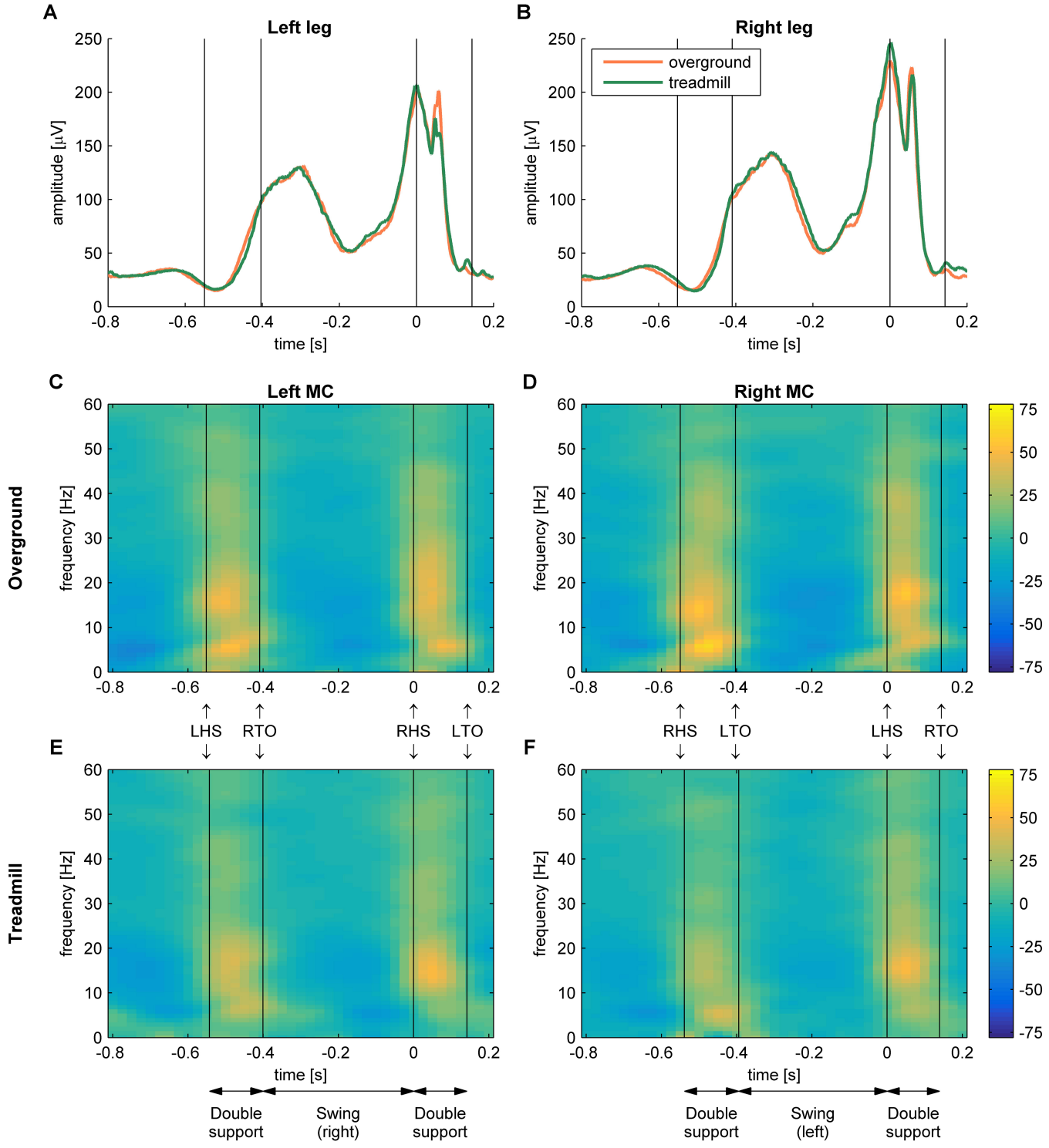
(A) Corticomuscular coherence between bipolar EEG from the motor cortex and contralateral TA EMG at the time point +50 ms (mid double support) during overground walking. Data were averaged across sides/channels (C3F3-TAr, C4F4-TAl). Grey patches indicate low beta frequency range (13-21 Hz), dots depict FFT frequency bins with significant CMC, horizontal dashed line shows upper limit of 95% CI. (B) Corresponding phase spectrum and fitted regression line in the lower beta band. (C) Estimated mean time lag between EEG and EMG in each frequency band (averaged across overground and treadmill, as statistical analyses identified no significant effect of gait condition on time lag). Bars and dots show the group mean; vertical lines depict the standard error of the mean. Time lags estimated based on slope of fitted regression line of individual phase spectra in each frequency band and condition. Positive time lag values indicate a time lag from EEG to EMG. CI, confidence interval; CMC, corticomuscular coherence.

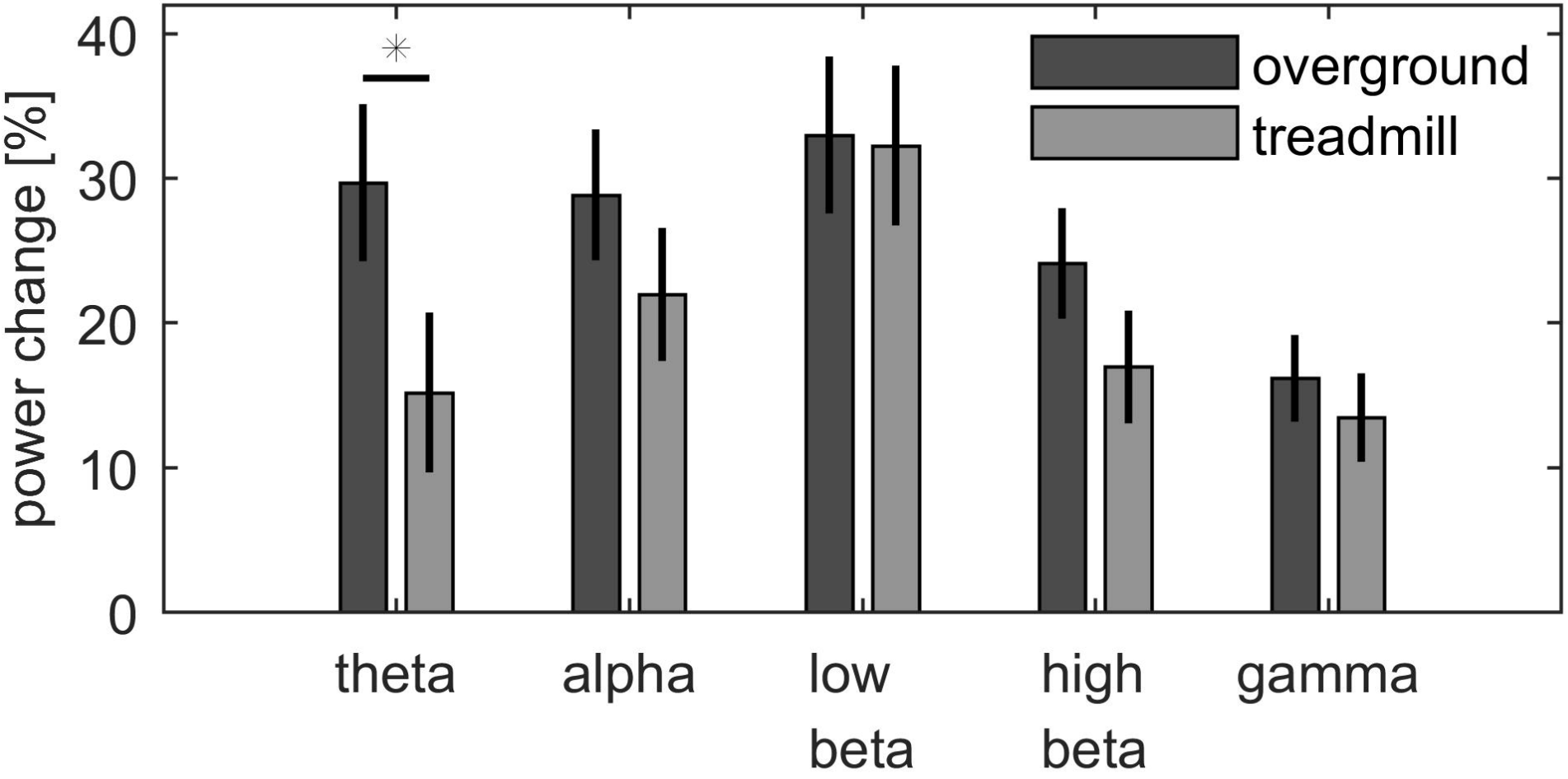
Tables

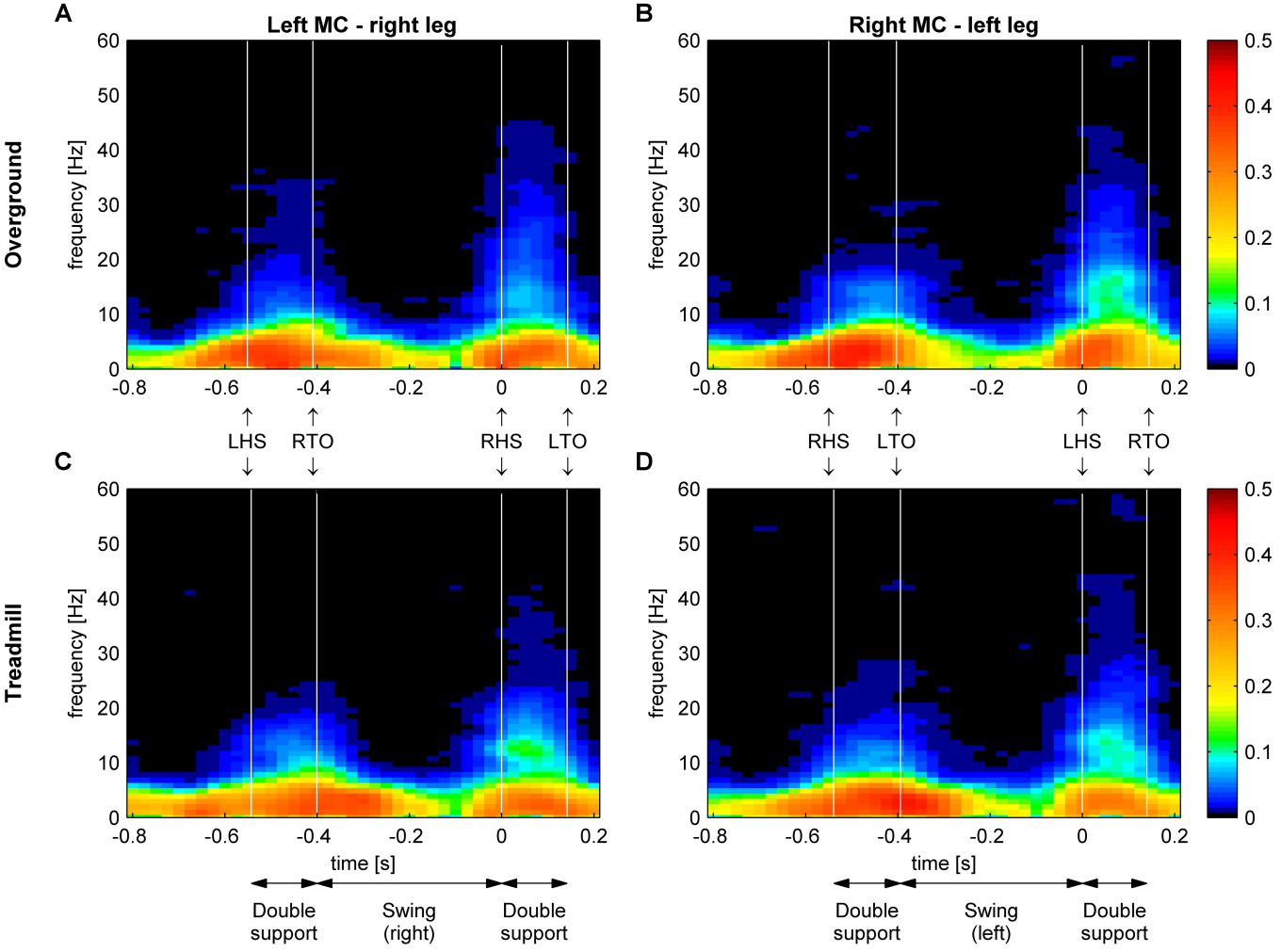
Table 1. Temporal gait parameters during overground and treadmill walking

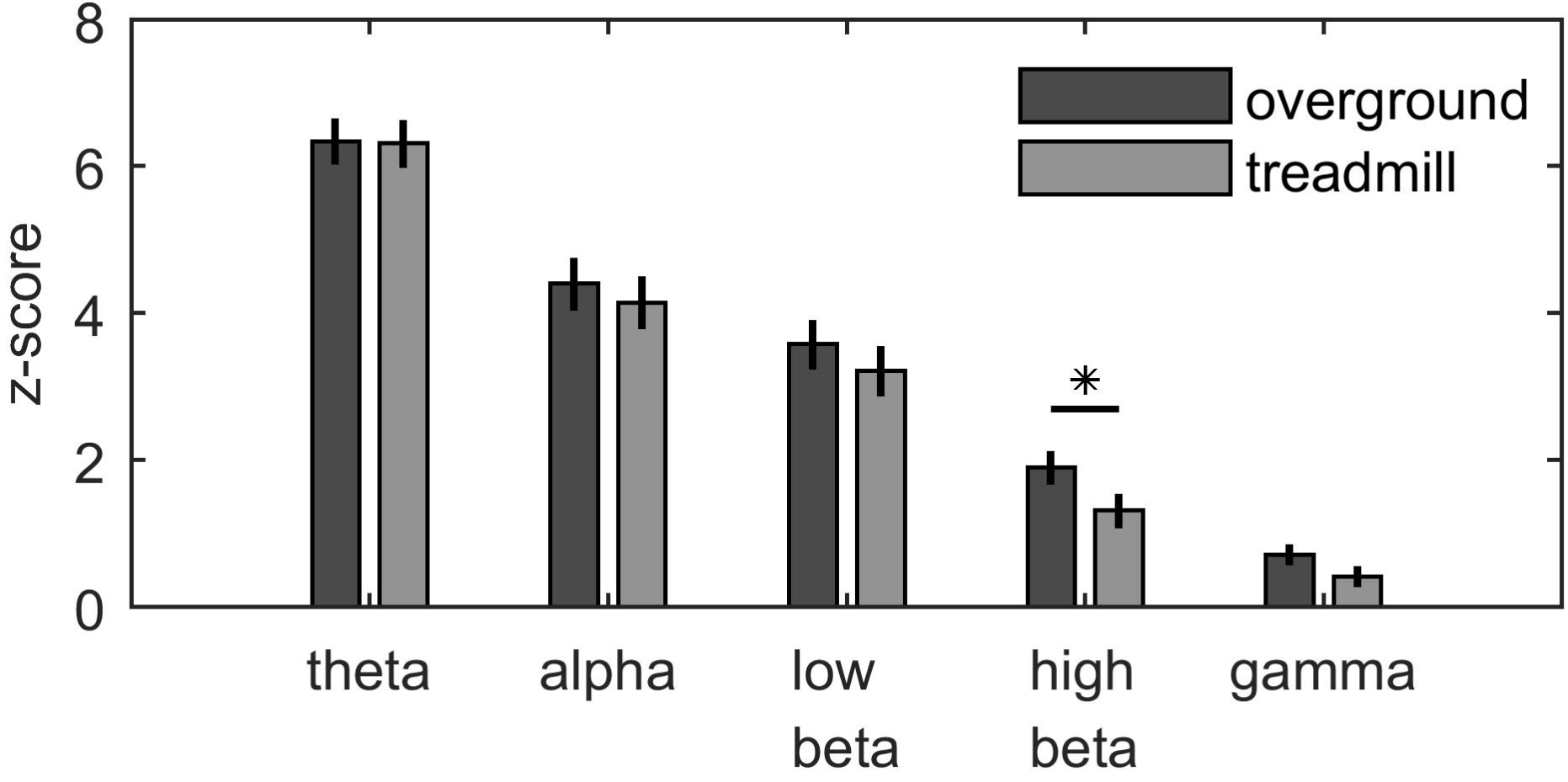
Gait Parameter	**Overground** mean (SD) n = 22	**Treadmill** mean (SD) n = 22	**T-statistic** T(df), p-value
Walking Speed (km h^{-1})	4.165 (0.411)*	4.178 (0.421)*	T(18) = -0.934, p = 0.362
Cadence (strides/min)	54.088 (3.817)	55.497 (3.440)	**T(21) = -2.750, p = 0.012**
Stride time (s)	1.115 (0.080)	1.085 (0.070)	**T(21) = 2.998, p = 0.007**
Stride time variability (s)	0.020 (0.006)	0.019 (0.019)	T(21) = 0.299, p = 0.768
Step time (s)	0.551 (0.040)	0.544 (0.042)	T(21) = 1.273, p = 0.217
Step time variability (s)	0.011 (0.004)	0.014 (0.021)	T(21) = -0.752, p = 0.460
Stance phase (s)	0.696 (0.072)	0.681 (0.060)	T(21) = 2.043, p = 0.054
Swing phase (s)	0.407 (0.024)	0.401 (0.032)	T(21) = 1.337, p = 0.196
Single support (s)	0.407 (0.024)	0.401 (0.032)	T(21) = 1.337, p = 0.196
Double support (s)	0.144 (0.036)	0.147 (0.046)	T(21) = -0.436, p = 0.667

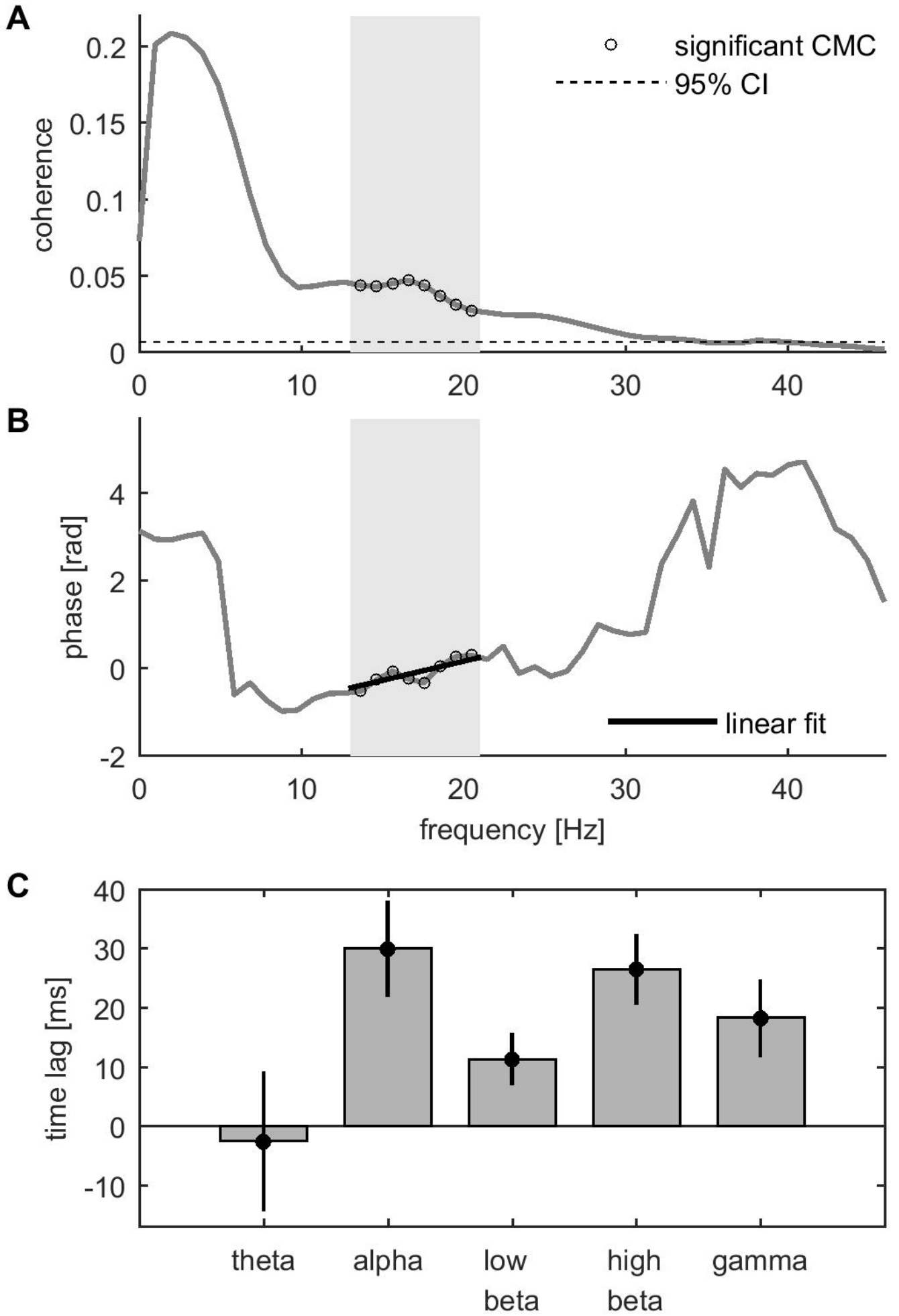
* n = 19


A
Left leg
B
Right leg
overground
treadmill
amplitude [μV]
time [s]
C
Left MC
D
Right MC
Overground
frequency [Hz]
LHS
RTO
RHS
LTO
E
F
Treadmill
Double support
Swing (right)
Swing (left)


40
30
20
10
0
power change [%]
*
overground
treadmill
theta
alpha
low beta
high beta
gamma


A
Left MC - right leg
B
Right MC - left leg
C
D
Overground
Treadmill
frequency [Hz]
time [s]
LHS
RTO
RHS
LTO
Double support
Swing (right)
Swing (left)


8
6
4
2
0
z-score
overground
treadmill
*
theta
alpha
low beta
high beta
gamma


A
significant CMC
95% CI
coherence
0.2
0.15
0.1
0.05
0
0
10
20
30
40
B
phase [rad]
4
2
0
-2
linear fit
frequency [Hz]
C
time lag [ms]
40
30
20
10
0
-10
theta
alpha
low beta
high beta
gamma