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# Learning to balance unilaterally: Synchronization in the cortico-spinal drive

Vrije Universiteit Amsterdam Faculty of Behavioural and Movement Sciences Qualification: MSc in Human Movement Sciences Research internship Research Master

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"I am always doing that which I cannot do, in order that I may learn how to do it..."

~ Pablo Picasso ~



#### Summary

Cortico-muscular  $\gamma$ -band oscillations (30-60 Hz) describe synchronized activity between the sensorimotor cortex and muscles and is believed to play a pivotal role in dynamical tasks. Short-term modulation of the cortico-muscular  $\gamma$ -band oscillations can be expected in a unilateral balance task because unilateral balance performance improves at a behavioural level as a function of short-term learning. Here, we targeted how cortico-muscular coherence (CMC) modulates in unilateral balance tasks across a balancetraining program. We expected that  $\gamma$ -band CMC to alter as a function of short-term motor learning and behavioural performance changes. 20 young healthy adults performed balance tasks pre- and posttraining. Balance tasks were performed on wooden boards (continuous tasks) and a robot-controlled platform (perturbation-induced). 64-channel electro-encephalography, electromyography of 36 muscles, and kinetics were collected.  $\gamma$ -band CMC between the sensorimotor areas and shank muscles of the stance leg significantly decreased post training in the perturbation-induced condition, while  $\gamma$ -band CMC in the continuous balance tasks was not modulated. Mean centre-of-pressure (COP) velocity decreased significantly post training in the continuous conditions while mean centre-of-mass (COM) velocity did not show this. No significant behavioural changes, mean COM-velocity and head acceleration, were found in the perturbation-induced condition. Significant Pearson's correlations coefficients indicated weak-tomoderate positive relationships between  $\gamma$ -band CMC and mean COP- and COM-velocity. CMC and behavioural changes as a result of the balance-training program showed no significant relationships. Perturbation-induced  $\gamma$ -band CMC decreased while continuous  $\gamma$ -band CMC did not change, suggesting task-dependent modulation during short-term balance learning.

**Key words:** motor control; neural oscillations; cortico-muscular coherence; sensorimotor function; balance tasks; short-term learning

**Abbreviations:** CNS: central nervous system; EEG: electro-encephalography; EMG: electromyography; CMC: cortico-muscular coherence; ERP: event-related potential; CI: confidence interval; AP: anteriorposterior; ML: mediolateral; PCA: principal component analysis; ICA: independent component analysis; ECG: electrocardiogram; MRI: magnetic resonance imaging; DICS: dynamic imaging of coherent sources; CSD: cross-spectral density; PSD: power spectral density; TFR: time-frequency representation; COM: centre-of-mass; COP: centre-of-pressure; LME: linear mixed-effects (model); REML: restricted maximum likelihood; GLME: generalized linear mixed-effects (model); ANOVA: analysis-of-variance; AIC: Akaike information criterion; BIC: Bayesian information criterion; RMS: root-mean-squared; TA: tibialis anterior; PN: peroneus longus; NNMF: non-negative matrix factorization



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# 1 Introduction

The maintenance of postural balance is a complex sensorimotor task as it involves the processing and regulation of multisensory information and requires an accurate muscular activation (Taube and Gollhofer, 2011; Torres-Oviedo and Ting, 2010). Improvements in postural balance, as reflected in reduced fall risk, can be accomplished with long-term balance-training programs (Halvarsson et al., 2013). Long-term balance programs may result in adaptations at the anatomical level (Taubert et al., 2010) and excitability (Taube et al., 2007) of the central nervous system (CNS). Healthy adults are even able to improve their unilateral balance performance by decreasing postural sway with a 30-minutes balance training (van Dieën et al., 2015). While this study by van Dieën et al. (2015) highlights the effectiveness of learning to balance for a short period on kinematic and behavioural level, the exact involvement and mechanisms of the CNS accompanying short-term improvements of the postural balance are still under debate (Jacobs and Horak, 2007).

In the CNS, neural populations interact with other populations to allow information flows from one group to the other. This neural communication within the multiple levels of the CNS is complex as it involves both spatial and temporal processing and regulation of the firing behaviour of millions of neurons (Kelso, 1997). While it is unclear how these specific control strategies are generated by the CNS, spatiotemporal coordination patterns within the CNS comprise synchronization between local and global neural populations in terms of periodic neural activity (Aumann and Prut, 2015). Such neural correlates have been observed between a widespread range of neural structures (Boonstra et al., 2009; Brown, 2000; Hori et al., 2013). However, the exact mechanisms and functions of this oscillatory behaviour remain to be explored. Nonetheless it is common sense that these neural oscillations play a prominent role during multisensory and sensorimotor information processing across the sensorimotor system (Engel and Fries, 2010).

Neural oscillations between the sensorimotor cortex and muscles have been observed in a wide-spread range of motor control tasks (de Vries et al., 2016; Gwin and Ferris, 2012; Kristeva et al., 2007; Murnaghan et al., 2014; Perez et al., 2006; Witham et al., 2010). Again, they may subserve a role in information processing (Donoghue et al., 1998). The neural connectivity between the cortical regions and muscles can be estimated by cortico-muscular coherence (CMC), which is quantified by the normalized cross-spectrum between co-registered electro-encephalography (EEG) and electromyography (EMG) (Baker and Baker, 2003). CMC describes a linear relationship between neural oscillations in ascending and descending cortico-spinal tracts originating from the summated activity of pyramidal neuron populations at cortical levels and spinal motoneural populations at the muscular level (Baker et al., 1999; Witham et al., 2011). The cortico-spinal oscillations may be a direct consequence of phase-locked circuits along the sensorimotor tracts (e.g., fast pyramidal pathways) involving the discriminatory activation of the individual muscles (Lemon, 2008) or synergistically activated neighbouring muscles (Reyes et al., 2017).

Movement-related paradigms regarding the modulation of cortical power (amplitude-based local synchronization) and cortico-spinal synchronization (phased-based global synchronization, e.g., CMC) has mainly been investigated in the  $\beta$ -band (15-30 Hz) and compromise static upper-body tasks. Steady-state periods of sustained contractions (e.g., isometric contractions) induce an upregulation of the local and global synchronization in the  $\beta$ -band, while (dynamic) movement preparation and execution abolish

this  $\beta$ -band synchronization (Baker et al., 1997). Short-term task-practicing gives rise to transient modulation of the  $\beta$ -band CMC, resulting in significantly higher  $\beta$ -band CMC after the learning period (Larsen et al., 2016). This modulation of  $\beta$ -band cortico-muscular phase synchronization with learning is correlated to improvements in the static manual motor performances at a behavioural level as well (Houweling et al., 2010; Kilner et al., 2000). The current idea regarding the modulation of  $\beta$ -band CMC is that these cortico-muscular oscillations may modulate during short-term task-practicing because of the "binding" process of novel information along the cortico-spinal axis (Lemon, 2008). For example, an upregulation of the cortico-spinal drive due to task learning may correspond to upweighting of sensory reafference via visual, proprioceptive (e.g., muscle spindles), or cognitive tracts (Baker, 2007). The existence of  $\beta$ -band CMC is inter-individually dependent but correlates to the rate of task performance improvement (Ushiyama et al., 2017).

While most experimental studies concern  $\beta$ -band CMC during static precision tasks, the occurrence of  $\gamma$ band (30-60 Hz) CMC in motor control is more enigmatic.  $\gamma$ -band CMC has shown to be different in synchronization properties and spatial topology compared to  $\beta$ -band CMC (Kopell et al., 2000; Mehrkanoon et al., 2014). Neural  $\gamma$ -band oscillations have mainly been observed during dynamical motor control tasks, such as locomotion (Petersen et al., 2012) and manual movements (Mendez-Balbuena et al., 2011; Omlor et al., 2007). Since  $\gamma$ -band CMC has been associated with dynamical motor performance, it has been proposed that CMC in this band is related to movement correction, occurring after a prediction mismatch between the expected and real sensorimotor performance (Schoffelen et al., 2005). Expectedly, the degree of movement errors in, for example, novel postural control tasks, would decrease as a function of practice and cortico-muscular oscillations in the  $\gamma$ -band would decrease as well. However, it remains unclear how  $\gamma$ -band CMC changes across a balance-training program, as this has never repeatedly been measured across motor learning.

The main objective of the current study was to investigate the short-term modulation of global neural  $\gamma$ -band (30-60 Hz) synchronization during unilateral balance tasks. The **overall hypothesis** was that the long-range  $\gamma$ -band synchronization in the cortico-spinal drive modulates as a function of short-term learning in both ongoing oscillatory and event-related unilateral balance tasks. It was expected that (changes in) cortico-muscular  $\gamma$ -band oscillations correlate to (changes at) behavioural level (e.g., kinematic and kinetic parameters) as well.  $\gamma$ -band CMC was studied before (pre-timepoint) and after (post-timepoint) a short-term learning period of a novel dynamical full-body task. This novel dynamical full-body performance consisted of unilateral surface stability tasks at stable and unstable balance platforms. Pre- and post-timepoint were separated by a balance-training program of 30 minutes, which consisted of non-perturbation-induced 30s-trials. Three unilateral surface stability tasks on different balance boards were studied. The first two consisted of balancing unilaterally on either a stable or an unstable balance board on which participants stood as quiet as possible. While participants balanced unilaterally for half-a-minute on the balance board, the continuous neural oscillatory behaviour was investigated. To contrast, the third balance board was a robot-controlled balance platform on which participants should anticipate adequately to imposed mechanical perturbations. In the latter case, CMC was related to the perturbations and concern event-related behaviour. Using this paradigm may also clarify whether the continuous or event-related  $\gamma$ -band CMC comprised different neural modulation. Learning effects were verified at behavioural level by the acquisition of full-body motion capture and measurement of ground reaction forces. To address possible neuro-physiological and behavioural interactions during learning,  $\gamma$ -band CMC was correlated to behavioural outcomes.

# 2 Methods & Procedures

# 2.1 Participants

Twenty young right-legged healthy participants (15 males and 5 females; mean±SD; age: 24.7±3.0 years; weight: 74.3±12.8 kg; height: 181.7±10.2 cm) were recruited at the Faculty of Behavioural and Movement Sciences. They were fellow students. They were included if they were aged between 18 and 35 years. Exclusion criteria were: recent troubles with injuries (less than 6 months), experienced pain or discomfort or any range of motion limitation during activities of daily living and exercises, and a history of neurological disorders or a history of participation in balance practicing sports (e.g., ballet, yoga, or gymnastics), as this may limit balance improvements during the balance-training program (Kiers et al., 2013). Participants signed an informed consent prior to performing the experiment. The local ethical committee of the Faculty of Behavioural and Movement Sciences, Vrije Universiteit Amsterdam (VCWE-2016-200) approved the experiment.

After extended pilot testing with five participants, a sample size calculation was performed using a dependent T-test in G\*power 3.1 (Universität of Düsseldorf, Germany). An effect size of 0.80 was established based on the differences in the means and standard deviations between the pre- and post-timepoint in CMC-estimates in the frequency band of 30-60 Hz. Alpha-level and power were 0.05 and 0.95, respectively. This a-priori power analysis showed that a sample size of 19 participants was necessary to reject the null hypothesis that the means of CMC within the frequency band of 30-60 Hz were equal between pre- and post-timepoint. Accordingly, 20 people were recruited to participate in the present study.

# 2.2 Experimental paradigm

We used a longitudinal study design with intra-subject pre-post comparisons. All measurements were conducted at the Vrije Universiteit Amsterdam. The measurement protocol consisted of a pre-timepoint, balance-training program and post-timepoint (Figure 1). At the pre- and post-timepoints, three unilateral balance tasks were executed on stable, unstable, and robot-controlled boards. Prior to the protocol, two reference trials were conducted: sitting on a chair and quiet standing on a force plate. Both reference trials were performed with open eyes and lasted 60 seconds. Stable and unstable board trials were performed on both legs and the robot-controlled board on the (dominant) right leg, resulting in five conditions: left stable, right stable, left unstable, right unstable, and robot-controlled. Subjects stood unilaterally on a stable or unstable wooden board during the stable and unstable board tasks, respectively (Figure 2A and B). Participants performed unilateral balance tasks on the right and left leg alternately until they performed five trials on both legs. A trial lasted 30 seconds. Participants had a break of 30 seconds between consecutive trials in order to prevent fatigue. Prior to every condition, participants performed one familiarization trial of 30 seconds on the balance board.





**Figure 1.** Schematic representation of the measurement protocol. 'Pre-timepoint' and 'Post-timepoint' both consist of five experimental conditions: 'left stable', 'right stable', 'left unstable', 'right unstable', and 'robot-controlled'. The two timepoints were intermitted by a balance-training program of 30 minutes. Training and board effects of the stable and unstable board were determined based on the average of five trials per timepoint and condition, respectively. Training effects of the robot-controlled balance board were investigated by the average of ten trials (330 perturbations) per timepoint.

In the third balance task, rotational perturbations were imposed on the axis of the robot-controlled balance board (Figure 2C). Participants performed ten trials of 30-40 perturbations on the right leg, comprising 330 perturbations in total. Perturbations had a magnitude of +3° or -3° and were imposed at a rate of  $\pm 1$  per second, resulting in single trial duration of  $\pm 40$ s. Extensive pilot measurements were executed to verify the number of trials, amount of perturbations, perturbation magnitude, intra- and inter-perturbation time in relation to the behavioural and neuro-physiological outcomes, such as the positive and negative potential characteristics of the event-related potential (ERP) (e.g., N200 and P300). To determine the number of perturbations a bootstrapping method was applied on pilot results (see Appendix B). The 95% confidence interval (CI) was estimated based on the random selection of the time-locked potential at Cz of 2-380 perturbations. This estimation procedure of the random selection of potentials was repeated for 5,000 times. The resulting exponential decay showed that the imposition of 320 perturbations would lead to an increase of <5% of the 95% CI,  $CI_{95\%}$ =[-0.250; 0.287 µV], compared to the estimated 95% CI of the grand average ERP based on 380 perturbations,  $CI_{95\%}$  = [-0.240; 0.276  $\mu$ V]. Perturbations were sinusoidal and had a magnitude of +3° or -3°. The board angle changed with an angular velocity was equal to  $+6^{\circ}$ /s or  $-6^{\circ}$ /s for 0.5s, reached the maximal value of  $+3^{\circ}$  or  $-3^{\circ}$ , and returned to its equilibrium. Pilot measurements showed that with these perturbations parameters people did not tend to grab to the hand rails next to the robot-controlled balance platform.

On every of the three balance boards, participants were instructed to place one foot parallel to attached tape on that particular balance board. Participants stood in socks on the balance boards, because proprioceptive signalling pathways of the feet is one of the main pathways associated with balance control (Mohapatra et al., 2014). When participants stood balanced unilaterally, they were instructed to place their arms akimbo, stand as quite as possible and try to avoid stepping off the board. In the robot-controlled balance task, participants were instructed to anticipate adequately on every perturbation and stabilize their body at the pre-perturbation equilibrium. During all conditions, they were also asked to fixate their gaze at a white cross of 10x10 cm on the wall at eye level at a distance of five meter, as head and eye movements may introduce excessive movement artefacts to the EEG. Participants were allowed to use their arms to maintain balance, since the arms are essential to regulate angular momentum in balance. A trial was considered as invalid when the participant stepped off the balance board or grabbed one of the chairs next to them in order to prevent falling.



Pre- and post-timepoints were intermitted by a balance-training program. In this balance-training program of 30 minutes, participants practiced their unilateral balance on two boards on both legs. These balance boards varied in height in order to ensure that the balance task became more challenging (Sherrington et al., 2008). While practicing on different balance boards is recommended (Kümmel et al., 2016), standardization of the intensity, duration, and volume of the balance-training programs is currently lacking except for general guidelines (Lesinski et al., 2015; Taube and Gollhofer, 2011). Accordingly, participants should practice 4-6 sets for 30 seconds unilaterally at every board separated by 30s pauses followed by the same procedure with the other leg. Therefore, in the current study, 5 unilateral trials of 30 seconds were conducted with 30s rest between trials on every balance board. Between the two boards, participants got a rest of 5 minutes to avoid fatigue.



Figure 2. Balance boards as used in the current study. A: Stable board. B: Unstable board. C: Robotcontrolled balance platform.

#### 2.3 Data acquisition

# 2.3.1 Balance board specifications

See Table 1 for the width surface, radius cylinder, and board height of the balance boards. The stable board (Figure 2A) consisted of a block of wood that was not able to rotate or translate and was comparable with a rigid solid ground. The unstable board (Figure 2B) had been constructed as a partial cylinder, creating an unstable surface around one axis (one degree of freedom) with a flat surface on top of the cylinder. The unstable board was constrained to rotate around the anterior-posterior (AP) axis, because controlling the medial-lateral (ML) sway seemed cortically and muscularly the most challenging task to maintain balance (Kelly et al., 2012; Slobounov et al., 2008). With the robot-controlled balance platform (Figure 2C) (HapticMASTER, MOOG B.V., Nieuw-Vennep, The Netherlands) mechanical perturbations can be exposed and regulated externally. The footplate of the robot-controlled balance platform can rotate around an axis beneath the participant. In the current study, position-based perturbations (e.g., board angle) were selected to manipulate the axis beneath the robot-controlled balance balance board. Board angle of the balance platform were lacking, validity and reliability of the platform were investigated extensively. Intra-class correlation coefficients were computed between the time series

containing the same perturbations to assess the reliability. Intra-class correlation coefficients were ranged between 0.999 and 1.000, implying that perturbations were completely reproducible.

Board name	Width surface (cm)	Radius cylinder (cm)	Height of board (cm)
Stable board	40.0	N/A	10.0
Unstable board	20.0	24.0	13.5
Robot-controlled board	11.3	11.3	24.0
Training board 1	20.0	24.0	13.5
Training board 2	20.0	24.0	18.5

**Table 1.** Mechanical characteristics of the balance boards as used during the measurement protocol.

Participants were prepared with EEG, EMG, and kinematic equipment by two researchers in order to reduce preparation time. EEG, EMG, kinematic, and kinetic data were acquired during each trial at the pre- and post-timepoints. The start of every trial was marked by a trigger and was used for offline alignment of EEG, EMG, kinematic, and kinetic time series.

# 2.3.2 Electro-encephalography

Participants wore a 64-electrode EEG cap. The EEG cap was placed according the international 10-20 standard. Impedance gel (SonoGel, Bad Camberg, Germany) was injected to improve the impedance between the skin and every electrode with a threshold of 10 k $\Omega$  during recordings. Every channel was recorded and 24-bits analogue-to-digital converted at 2,048 samples/s by a TMSi Refa 128-channel amplifier (TMSi, Twente, The Netherlands). An average common reference of the 128 channels (64 EEG channels and 64 unipolar EMG channels) was applied as reference. Prior to measurements, participants were asked to avoid eye blinking, jaw clenching, head movements, or facial expressions that could introduce artefacts into the EEG signals.

# 2.3.3 Electromyography

Surface EMG activity of tibialis anterior, peroneus longus, gastrocnemius medialis, soleus, rectus femoris, vastus lateralis, biceps femoris, adductor longus, gluteus medius, rectus abdomis, external oblique, longissimus, iliocostalis, latissimus dorsi, pectoralis major, trapezius descendens, deltoid medius, and sternocleidomastoideus were bilaterally measured, resulting in the acquisition of 36 muscles. Activity of these muscles had been acquired as it is thought that they play a major role in balance. EMG time series were acquired with the remaining unipolar (64) and bipolar (4) channels of the Refa 128-channel amplifier, equalling the collection of the activity of 18 muscles bilaterally. Ag/AgCl surface electrode pairs (Medicotest, Ambu® Blue Sensor®, type: N-00-S/25) were attached according SENIAM conventions (Hermens et al., 2000). The inter-electrode distance was about 2.0 cm. As EEG and EMG were acquired with the same amplifier, the ground channel of the EEG had been used as reference for the unipolar EMG instead of placing reference electrodes.

# 2.3.4 Kinematics and kinetics

Full-body kinematics were acquired with one Optotrak camera (Northern Digital, Waterloo, ON, Canada), which was placed posterior to the subject. Seventeen three-dimensional single LED markers were placed on the heels (proximal part of the calcaneus), knees (posterior between medial and lateral condyles), hips (left and right SIPS), shoulders (posterior part acromion), elbows (medial epicondyle and olecranon), wrists (ulnar and radial styloid processes), and neck (spinous process level C7) to obtain

their 3D-positions with a sampling frequency of 150 samples/s. Two single LED markers were attached to each elbow and wrist to ensure that at least one marker was visible during the experiments.

GRFs were measured with a customized force platform, which is made by the Department of Human Movement Sciences of the Vrije Universiteit in Amsterdam. The signals of the force platform were amplified and sampled by a sampling frequency of 200 samples/s.

# 2.4 Data pre-processing

All analyses were performed with the software of MATLAB 2015b (Mathworks Inc., Natick, USA). In addition, the open-source FieldTrip Toolbox was used for pre-processing and artefact detection (http://fieldtrip.fcdonders.nl/) (Oostenveld et al., 2011). Pre-processing started with the re-referencing of the EEG and derivation of bipolar EMG signals, because an average common reference was applied across all 128 unipolar channels of the Refa amplifier while 64 EEG and 64 unipolar EMG channels were measured simultaneously. EEG signals were re-referenced to an average common reference and one bipolar EMG derivation was determined out of every two unipolar EMG derivations. Principal component analysis (PCA) and independent component analysis (ICA) were consecutively applied to remove the contamination of the electrocardiogram (ECG) from the bipolar EMG signals, since the frequency content of the EMG signals is retained after the application of these multivariate statistical approaches (Willigenburg et al., 2012). PCA and ICA decompose multivariate datasets of time series into sets of perpendicularly uncorrelated and statistically uncorrelated modes, respectively (Jutten and Herault, 1991). The first PCA-mode was consistently contaminated with ECG and was hence removed. The number of removed ICA-modes ranged between 2 and 8 modes. ICA-modes were inspected visually and removed manually when they were considered as ECG-contaminated.

The EEG and EMG time series were de-meaned, and band-pass filtered (bidirectional second-order Butterworth filter between 5 and 250 Hz). Line noise and its higher harmonics were removed by a notch filter (bidirectional second-order Butterworth around 50, 100, 150, 200, and 250 Hz with a bandwidth of 1 Hz). The EEG time series were temporarily re-referenced to channel F7 to detect bad channels. EEG channels were qualified as 'bad' in case the channel contained a flat line or a too high or low amplitude. Channels were excluded based on amplitude when the variance of a single channel exceeded 3 times the standard deviation or 10 times the maximum of all channels. On average, 0.7 channels (range: 0-5 channels) were removed in every trial. The activity of the bad EEG channels was interpolated with a spheric spline interpolation based on the activity of the neighbouring channels and electrode positions of the channels. After checking bad channels, EEG time series were re-referenced to an average common reference again. Advanced artefact detection and removal was performed with ICA. ICA was applied on the EEG time series using the FieldTrip Toolbox functions including an extended fastica algorithm with online bias adjustment. Single ICA-modes were removed when the median frequency of that particular mode was lower than 1 Hz or higher than 60 Hz. Finally, the activity of left and right sternocleidomastoids were implemented in an additional PCA to reduce the muscular artefacts in the EEG time series.

Rectified EMG signals were obtained by taking the modulus of the complex-valued analytic signal, because rectification provides the summated temporal firing pattern of groups of motor units independent of their action potential shape (Boonstra and Breakspear, 2012). In CMC-estimates based on sensor-level EEG, EEG time series were adjusted to reference-free current source density derivations

using spherical spline with default parameters in FieldTrip regarding conductivity, lambda and order. The application of a Laplacian derivative improves the subsequent EEG-EMG coherence estimates (Mima and Hallett, 1999), because of the spatial filtering effect of current source density around a unique EEG channel.

# 2.5 Data analysis

# 2.5.1 Source reconstruction

After the analysis at sensor-level, cortical sources revealing maximal power modulation between preand post-timepoint around a predefined frequency (e.g., 40 Hz) were identified by the application of dynamic imaging of coherent sources (DICS) beamformers (Groß et al., 2001). The application of this type of spectral beamformers may offer an advantage for the consecutive CMC-estimates. To do so, a head model, lead field, and cross-spectral density (CSD) matrix were estimated. The head model was constructed using the magnetic resonance imaging (MRI) templates implemented in FieldTrip, because the participants' anatomical MRI data had not been collected. Default parameters of the shape of different head tissues were used to construct the head model. Admittedly, the source estimation accuracy would have increased if this head model could have been constructed with the individual participant's MRI. A lead field model was constructed based on a default template that is implemented in FieldTrip, three-dimensional electrodes' positions, and three-dimensional grids. The lead field resulted in a matrix describing the physical propagation of the electrical activity from the current sources to the channels at the surface of the EEG-cap. The CSD-matrix was estimated between all Fourier-transformed EEG signals using a multitaper Fourier transform with a 2s Hanning window. The CSD-matrix computation was centred around the frequency of 45 Hz, because bivariate CMC-estimates pinpointed significant CMC between 30-60 Hz. This CSD-matrix was pooled over consecutive windows within the EEG signals of every individual trial. Spatial filters were applied to estimate the reconstructed source signals.

The resulting reconstructed sources were compared using a cluster-based permutation test. The clusterbased permutation test is a non-parametric test, which does not require probability distribution assumptions and can solve the multiple comparisons problem occurring in voxel-matrices (Maris and Oostenveld, 2007). Cluster-based permutation tests were used for within-subject statistics for the statistical assessment of cortical areas showing  $\gamma$ -band power (i.e., 30-60 Hz) modulation between preand post-timepoint. Significant voxels yield the cortical areas of the sources, which explained significant  $\gamma$ -band power modulation. The permutation p-value of the permutation distribution was approximated with Monte Carlo estimation by repeating random partitions for  $2*10^{13}$  times. Cluster-alpha and criticalalpha for paired T-statistic were set at 0.00001 and 0.025, respectively. The reconstructed cortical source with significant  $\gamma$ -band power modulation between preand post-timepoint was selected. Right and left hemispheric virtual source signals based on the CTF-coordinates were decomposed by singular value decomposition giving the x-, y-, and z-direction of the significant reconstructed signal that explained most variance (e.g., the largest temporal eigenvector). This most prominent mode of both virtual source signals was consequently used as EEG time series for the CMC-estimates.

# 2.5.2 Spectral analysis

Power spectral densities (PSD) of the EEG channels of the sensorimotor cortex and the rectified EMG of the shank muscles channels for CMC-analysis were computed according Welch's segmentation method

including a Hamming window of 0.50 seconds with an overlap of 50% between different windows (Welch, 1967). EEG and EMG PSD spectra were logarithmically transformed to stabilize normality (Halliday et al., 1995). For connectivity analysis, the rectified EMG and EEG signals were spectrally transformed, also following Welch's segmentation approach. Auto-spectra and cross-spectra of the EEG and EMG time series were estimated using Fourier-transformed epochs of 0.50 seconds with a Hamming window with an overlap of 50% between consecutive windows. Hence, CMC was estimated between the 'beamformed' EEG and multiple (e.g., synergetic) rectified EMG signals. In general, coherence is defined as the modulus of the cross spectrum between an EEG and EMG signal divided by the square root of the product of both power spectra and was estimated accordingly:

$$Coh_{XY}(\omega) = \frac{\left|\sum_{i=1}^{N} X_i(\omega) Y_i^*(\omega)\right|^2}{\sum_{i=1}^{N} X_i(\omega) X_i^*(\omega) \sum_{i=1}^{N} Y_i(\omega) Y_i^*(\omega)}$$
(1)

where  $X_i(\omega)$  and  $Y_i(\omega)$  represent the Fourier transforms of an EEG and EMG signal at the *i*-th section as a function of frequency and \* denotes the complex conjugate. Magnitude-squared coherence spectra were Fisher's Z-transformed and consecutively averaged over trials and participants for every timepoint. The Fisher's transform assigned a constant value to the variance of the coherence to stabilize normality (Rosenberg et al., 1989). After pooling coherence of trials, and participants, the inverse Fisher's transform was applied in order to obtain a pooled CMC-estimate between 0 and 1. The  $\gamma$ -band (30-60 Hz) was the frequency range of interest, since significant  $\gamma$ -band CMC was observed in pilot experiments (Appendix A).

The 10 continuous EEG time series containing the 30-40 movement-related potentials of the robotcontrolled balance platform trials were time-locked into epochs of 1,000 ms after standard artefactremoval. Epochs were segmented from 400 ms before to 600 ms after perturbation onset. Epochs were consecutively pooled within and across trials and participants for both timepoints, resulting in an average of 4,950 movement-related potentials at pre- and post-timepoint. EEG and EMG time series were transferred to the frequency domain according a multi-taper frequency-convolution with a Hanning-taper as implemented in FieldTrip. Consequently, time-frequency representations (TFRs) of relative power and coherence were constructed.

# 2.5.3 Behavioural analysis

Trials were excluded from further analysis in case they contained missing values at the begin or end of time series creating instability during extrapolation. Furthermore, trials were not considered for analysis if these contained excessive variance, for example, in case, the participant grabbed the hand rails multiple times during a single trial. Missing values within the kinematic time series were interpolated using cubic splines. Kinetic signals were additionally low-pass filtered (bidirectional second-order Butterworth filter at 15 Hz). Kinematic and kinetic time series were averaged for all trials and subjects per condition and timepoint.

Two main outcomes of the kinematic and kinetic data were determined: mean centre-of-mass (COM) velocity and mean centre-of-pressure (COP) velocity, since these biomechanical parameters play a significant role in maintaining human balance (Hof, 2007). Although the unstable board could only rotate around the AP-axis, AP-sway was taking into consideration, since it has been shown that minimizing sway in a certain direction may result in an upregulation of the orthogonal direction (Balasubramaniam

et al., 2000). Mean COM- and COP-velocity in AP- and ML-direction were computed by taking the mean of the modulus of the time series of the COM- or COP-velocity in each direction (van Dieën et al., 2015). Since the trials consisted of a standardized duration the mean COM- and COP-velocity are equivalent to the mean COM- and COP-velocity, respectively. It has been suggested that both mean COM- and COP-velocity in ML-direction is an accurate and operational performance measure to assess the (gradual) skill acquisition during balance tasks (van Dieën et al., 2010).

# 2.6 Statistical analysis

# 2.6.1 Statistics of neurophysiological data

CMC was pooled for trials and participants and considered as significant different from zero at a certain frequency when its value surpassed the 95% *CI*. The 95% *CI* was estimated as  $1 - (0.05)^{\sum L_i - 1}$  where  $L_i$  is the amount of windows (Halliday et al., 1995). The *CI* was adjusted to take into account the overlap between consecutive windows (Welch, 1967) and was set at 0.022.

The equality of the means of the coherence estimates at every frequency across pre- and posttimepoints was tested with the  $\chi^2$ -statistic as proposed by Amjad and colleagues (1997) (Amjad et al., 1997). The  $\chi^2$ -test statistic to assess the equality in the means of the coherence estimates as a function of frequency is:

$$\chi^{2} = 2 \left[ \sum_{i=1}^{k} L_{i} * \hat{z}_{i}^{2} - \left( \frac{\left( \sum_{i=1}^{k} L_{i} * \hat{z}_{i} \right)^{2}}{\sum_{i=1}^{k} L_{i}} \right) \right]$$
(2)

In which  $\hat{z}_i$  represents the *i*-th pair of the Z-scored modulus of the coherency,  $L_i$  the amount of disjoint sections, and *k* the amount of coherence estimates. Significance level of this  $\chi^2$ -statistic was set at the value  $\chi^2_{(\alpha;k-1)}$ . If a significant difference is indicated by the  $\chi^2$ -value then the CMC-spectra should be considered to see whether the CMC-modulation corresponds to an increase or decrease as a function of the balance-training program.

Cortico-muscular time-frequency coherence representations were compared with *Timepoint* as factor using cluster-based permutation tests with a dependent T-test as test statistic. CMC was pooled for consecutive perturbations within single trials. Like the beamforming statistics, Monte Carlo estimations with  $2 \cdot 10^{13}$  random partitions were used to estimate the probability distribution. The T-values of the cluster-based permutation test were thresholded for statistical significance and plotted as a function of frequency and time as well.

# 2.6.2 Statistics of kinematics and kinetics

Given the probability of correlations between the trials of the pre- and post-timepoints within a single participant and given performance variability between participants, a linear-mixed effects (LME) model was fitted in order to obtain more powerful repeated measures statistics. Four LME-models with different behavioural outcomes were fitted on the COM- and COP-data as acquired with the trials on the stable and unstable balance board. These four behavioural outcomes were: mean COM-velocity in (1) ML-direction, and (2) ML- and AP-direction, and mean COP-velocity in (3) ML-direction, and (4) ML- and AP-direction. The estimated LME-model was equal to:



 $Behavioural outcome = \beta_0 + \beta_1 Timepoint + \beta_2 Leg + \beta_3 Board + \beta_4 Leg \times Board + \beta_5 Leg \times Timepoint + \beta_6 Timepoint \times Board + \beta_7 Timepoint \times Leg \times Board + \gamma_0 + \gamma_1 Timepoint + \varepsilon$ (3)

in which the fixed effects are represented by the intercept ( $\beta_0$ ), *Timepoint* ( $\beta_1$ ), *Leg* ( $\beta_2$ ), *Board* ( $\beta_3$ ) and three two-way interactions between *Leg* and *Board* ( $\beta_4$ ), *Timepoint* and *Board* ( $\beta_5$ ), and *Leg* and *Timepoint* ( $\beta_6$ ), and a three-way interaction between *Timepoint*, *Leg*, and *Board* ( $\beta_7$ ). Random effects are  $\gamma_0$  and  $\gamma_1$ , including the random effect of the intercept and slope, respectively, across participants. Since the initial balance level of participants may influence possible balance improvements,  $\gamma_0$  and  $\gamma_1$  were allowed to be correlated. Finally,  $\varepsilon$  corresponds to the residual error term. The significance level was set at an alpha level of 0.05. The random factor variance of the intercept was estimated using restricted maximum likelihood (REML). In case a Gaussian distribution in the (logarithmically transformed) data was lacking as shown by a boxplot, a generalized linear mixed-effects (GLME) model was constructed. To investigate the mean COM-velocity response of the robot-controlled balance platform, a new LME-/GLME-model was created containing intercept ( $\beta_0$ ) and *Timepoint* ( $\beta_1$ ) as fixed effects and  $\gamma_0$  and  $\gamma_1$  as random effects. Mean COM-velocity of the robot-controlled balance platform was only analysed in the ML-direction, since perturbations were imposed in the ML-direction.

Finally, Pearson's correlations were computed between  $\gamma$ -band CMC and mean COP-velocity for every participant independent of timepoints. Furthermore, to investigate correlations associated with motor learning, Pearson's correlations were calculated between the difference in  $\gamma$ -band CMC and the difference in mean COP- and COM-velocity between pre- and post-timepoint.

# 3 Results

3.1 Behavioural results

#### 3.1.1 Stable and unstable board

А.



В.

**Figure 3.** Grand averages across trials and participants of the mean velocity of centre-of-pressure (COP) and centre-of-mass (COM) data. **A** and **B**: Mean COP- and COM-velocity, respectively, based on the absolute sway in anterior-posterior and mediolateral direction across timepoints, legs and board. Error bars indicate 1 standard deviation. **C** and **D**: Mean COP- and COM-velocity, respectively, based on the excursion in medial-lateral direction across timepoints, legs and boards. Note that the y-axes of **A** and **C** are different compared to **B** and **D**.

Figure 3 illustrates mean COP- and COM-velocity in AP- and ML-direction and in ML-direction. Mean COPand COM-velocity were logarithmically transformed, however, boxplots identified that Gaussian distributions were still lacking. Four GLME-models were constructed according *Equation 3*. The GLMEmodel of the mean COP-velocity in ML- and AP-direction revealed significant main effects of *Timepoint*, and *Board* and a significant two-way interaction of *Timepoint* x *Board* (Figure 3A). Hypothesis testing with F-tests revealed a significant effect of *Timepoint*,  $F_{(1,583)} = 3.95$ , p = .047 in which the posttimepoint (3.726±0.006 cm/s; mean±standard error of the mean) was associated with a lower mean COP-velocity compared to the pre-timepoint (5.008±0.010 cm/s). In addition, there was a significant effect of *Board*,  $F_{(1,583)} = 210.61$ , p < .001. The unstable board was related to a higher mean COPvelocity in ML- and AP-direction (6.255±0.009 cm/s) compared to the stable board (2.582±0.003 cm/s). There was a significant *Timepoint* x *Board* interaction whereby the mean COP-velocity in ML- and APdirection on the stable board decreased less between pre- and post-timepoint than on the unstable board,  $F_{(1,583)} = 25.48$ , p < .001. The descriptive statistics for the *Timepoint* x *Board* interaction were:  $2.805\pm0.006$  cm/s on the stable board at the pre-timepoint and  $2.356\pm0.005$  cm/s at the post-timepoint; and  $7.311\pm0.019$  cm/s on the unstable board at pre-timepoint and  $5.153\pm0.011$  cm/s at the post-timepoint.

The GLME-model of the mean COM-velocity in ML- and AP-direction (Figure 3*B*). revealed a significant main effect of *Board* and a significant two-way interaction between *Timepoint* x *Board*. F-tests indicated a significant effect of *Board*,  $F_{(1,661)} = 6.68$ , p = .010. Balancing unilaterally on the unstable board corresponded to an increase of  $0.330\pm0.002$  cm/s in mean COM velocity in ML- and AP-direction relative to the stable board. The *Timepoint* x *Board* interaction indicated that the mean COM velocity in ML- and AP-direction on the stable board decreased less than on the unstable board between the pre- and post-timepoint,  $F_{(1,661)} = 10.25$ , p = .001. Mean values for the *Timepoint* x *Board* interaction were:  $1.357\pm0.003$  cm/s on the stable board at the pre-timepoint and  $1.276\pm0.002$  cm/s at the post-timepoint; and  $1.790\pm0.005$  cm/s on the unstable board at pre-timepoint and  $1.486\pm0.004$  cm/s at the post-timepoint.

The GLME-model of the mean COP-velocity in ML-direction (Figure 3C) showed a significant main effect of Timepoint and Board, and significant two-way interactions effects of Leg x Board and Timepoint x Board. An analysis-of-variance (ANOVA) revealed a significant effect of Timepoint,  $F_{(1,583)} = 4.44$ , p =.036, whereby the post-timepoint (1.668±0.003 cm/s) was associated with a lower mean COP-velocity in ML-direction than the pre-timepoint (2.206 $\pm$ 0.005 cm/s). There was also a main effect of Board,  $F_{(1,583)}$  = 229.38, p < .001. Standing on the unstable board (2.747±0.004 cm/s) was associated with an increase of the mean COP-velocity in ML-direction compared to the stable board (1.155±0.001 cm/s). The significant two-way interaction of Leg x Board,  $F_{(1,583)} = 9.81$ , p = .002, revealed that the mean COPvelocity in ML-direction was lower while standing on the right leg on the stable board compared to balancing on the left leg on the unstable board. The mean COP-velocity in ML-direction was lowest while balancing on the right and left leg on the stable board  $(1.114\pm0.002, and 1.195\pm0.002 cm/s,$ respectively), followed by balancing on the right and left leg on the unstable board (2.337±0.007, and 3.151±0.010 cm/s, respectively). Furthermore, the significant two-way interaction of Board x Timepoint,  $F_{(1,583)} = 19.28$ , p < .001, indicated that the balancing unilaterally on the unstable board resulted in a higher decrease as a function of time in mean COP-velocity in ML-direction relative to the stable board. The descriptive statistics of the Timepoint x Board interaction were: 1.250±0.002 cm/s on the stable board at the pre-timepoint and 1.057±0.002 cm/s at the post-timepoint; and 3.214±0.010 cm/s on the unstable board at pre-timepoint and 2.301±0.006 cm/s at the post-timepoint.

Finally, there was a significant main effect of *Board* and a significant two-way interaction effect of *Timepoint* x *Board* according the GLME-model of the mean COM-velocity in ML-direction, as illustrated in Figure 3D. The significant effect of main effect of *Board*,  $F_{(1,661)} = 9.92$ , p = .002, shows that balancing on the unstable board resulted in an increase of the mean COM-velocity in ML-direction with 0.230±0.046 cm/s relative to the stable board. Besides, as indicated by the other three F-tests, balancing unilaterally on the unstable board over time resulted in a higher decrease in mean COM-velocity in ML-direction compared to the stable board,  $F_{(1,661)} = 10.11$ , p = .001, as indicated by a significant two-way interaction of *Timepoint* x *Board*. The mean COP-velocity decreased less on the stable board (0.052±0.002 cm/s) than the unstable board (0.227±0.003 cm/s) as a function of time.

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Α.

В.

**Figure 4.** Individual response curves of the **A**: mean centre-of-pressure (COP) and **B**: mean centre-of-mass (COM) velocity in anterior-poster (AP)- and mediolateral (ML)-direction across the two timepoints. Participants balanced unilaterally on the unstable board on their left leg. The means at both timepoints are the average of the five trials as performed at that particular timepoint and are represented by the big dots. Smaller dots show the participant's performance at an individual trial at this condition. Fitted least-squared lines are illustrated by the green lines and are based on the means of the pre- and post-timepoint. Same coloured error bars at timepoints is related to 1 standard deviation. Abbreviations P01–P20 correspond to the participant number. Note that kinetic data have not been acquired during the experiments of P01-P05. vCOP: COP-velocity; vCOM: COM-velocity.

Although significant main effects of the GLME-models were indicated by the F-tests, the response curves of Figure 4 illustrate that both initial COP- and COM-velocity in AP- and ML-direction and the change in COP- and COM-velocity as a function of training were not homogenous among participants. This heterogeneous initial balance level and balance response to the balance-training program indicates that there are inter-individual differences in the behavioural data. Giving the four constructed GLME-models, the null-hypothesis can be tested that the intercept and slope of the random effect *Subject* is significantly different from 0 ( $H_0: \gamma_0 \neq 0$ ). Since the 95% *CI* of the four GLME-models did not contain 0, the random effect *Subject* of both intercept and slope was significant in the four GLME-models (intercept; slope: COP-velocity in AP-ML:  $CI_{intercept,95\%}$ =[0.508; 1.161 cm/s];  $CI_{slope,95\%}$ =[0.153; 0.770 cm/s] and ML-direction  $CI_{intercept,95\%}$ =[0.524; 0.584 cm/s];  $CI_{slope,95\%}$ =[0.092; 0.311 cm/s] and COM-velocity ML-direction  $CI_{intercept,95\%}$ =[0.366; 0.408 cm/s];  $CI_{slope,95\%}$ =[0.017; 0.214 cm/s]).

The effect of the intercept and slope of Subject in the model (e.g., by the goodness of fit including the random effects of Subject) can be assessed by comparing the same GLME-model with and without the random intercept and slope of Subject. Consequently, it can be tested whether the behavioural response (either COP- or COM-velocity) was mainly related to changes of the fixed-effects (e.g., Timepoint) or whether the behavioural response was influenced by both fixed and random effects. These autocomparisons of the four GLME-models identifies that the Akaike information criterion (AIC), Bayesian information criterion (BIC), and log likelihood values of the four GLME-models with the random intercept and slope were smaller than the four GLME-models without the random intercept and slope, suggesting that the GLME-models with random effects of the intercept and slope better fit the COM- and COPvelocity data. Furthermore, according the small p-values (p < .001), the null-hypothesis that the COMand COP-velocity response was only caused by fixed effects (e.g.,  $H_0$ :  $\gamma_0 = 0$ ) is rejected, which favours the alternative hypothesis that the COM- and COP-velocity were a function of the random effects of Subject. Note that although the p-values of the random effect intercept and slope identify a significant effect, the alternative hypothesis ( $H_1: \gamma_0 \neq 0$ ) cannot be confirmed based on this statistical test. Finally, it also appeared that the four GLME-models with and without the random effect of the Subject slope led to lower AIC, BIC, and log likelihood values of the GLME-model with the random slope of Subject and significant p-values (p < .001), rejecting the null-hypothesis that the model containing fixed effects and random intercept of Subject were only responsible for the COM- and COP-velocity.

# 3.1.2 Robot-controlled balance platform

Mean COM-velocity and root-mean-squared (RMS) head acceleration in ML-direction were not distributed according Gaussians. GLME-models were created containing intercept ( $\beta_0$ ) and *Timepoint* ( $\beta_1$ ) as fixed effects and  $\gamma_0$  and  $\gamma_1$  as random effects. The GLME-models of mean COM-velocity and RMS head acceleration showed no main effect of Timepoint (Figure 5). Likewise, F-tests showed no significant effect of *Timepoint* for mean COM-velocity and RMS head acceleration.



**Figure 5.** Grand average across trials and participants of the **A**: mean centre-of-mass (COM)-velocity and **B**: root-mean-squared (RMS) head acceleration of the balance task at the robotic-controlled balance platform. Mean COM-velocity and RMS of the head acceleration are based on the mediolateral sway.

# 3.2 Neuro-physiological results

# 3.2.1 Source localisation

When using the virtual source signals found with DICS-beamforming to estimate CMC with rectified EMG signals, the significant  $\gamma$ -band peak as found in the CMC-estimates containing the sensor-level EEG disappeared. Hence, sensor-level EEG time series were used to estimate CMC. The beamformer results based on significant  $\gamma$ -power modulation are addressed in Appendix C.

# 3.2.2 Continuous oscillatory results of the stable and unstable board

The pooled CMC spectra between sensor-level mid-central EEG channels (channel Cz) and contralateral rectified EMG signals of the shank muscles in the four continuous conditions are presented in Figure 6. Significant  $\gamma$ -band CMC was observed between the sensor-level EEG (channel Cz) and the contralateral tibialis anterior, peroneus, soleus, and biceps femoris. Significance of  $\gamma$ -band CMC as estimated with the leg muscles was only found in the stance leg while CMC-estimates with the EMG time series of the non-stance raised leg were not significant. In addition, significant  $\gamma$ -band CMC was mostly found between channel Cz and the left and right external oblique, rectus abdomis, gluteus medius, longissimus, iliocostalis, and deltoid medius. This significant  $\gamma$ -band CMC was observed at both pre- and post-timepoints. CMC-estimates between channel Cz and the remaining muscles (gastrocnemius medialis, rectus femoris, vastus lateralis, adductor longus, latissimus dorsi, pectoralis major, trapezius descendens, and sternocleidomastoideus) did not show significant  $\gamma$ -band CMC. In addition to the significant  $\theta$ -band CMC with the highest peak around 6 Hz. None of the CMC-estimates between the sensorimotor cortex and any of the muscles yielded significant CMC in the  $\beta$ -band.









Figure 6. Grand averages across trials and participants of the magnitude-squared cortico-muscular coherence spectra between the sensor-level EEG and the rectified EMG of the right and left tibialis anterior and peroneus in the A: left stable, B: right stable, C: left unstable, and D: right unstable condition, respectively. Pretimepoint is marked in light-blue and post-timepoint in dark-red. Coloured shaded surfaces represent 1 standard deviation. The horizontal grey lines correspond to the 95% confidence interval and was set at 0.022.

The occurrence of  $\gamma$ -band CMC was very heterogeneous across participants independently of the modulation across timepoints. Significant  $\gamma$ -band CMC was detected in eight out of the 20 participants, which usually were CMC-estimates based on the muscles showing significant  $\gamma$ -band CMC at grand average level as well. However, as on grand average level, the occurrence of the significance of  $\gamma$ -band CMC in individuals differed between muscles and tasks.

To statistically evaluate the  $\gamma$ -band CMC-modulation as a result of the balance-training program, EEG and EMG signals of pre- and post-timepoint were statistically compared using the difference in coherence test. Figure 7 shows the  $\chi^2$ -test statistic of the CMC-estimates confirming significant  $\gamma$ -band CMC as evaluated according the difference in coherence test.  $\gamma$ -band CMC is significantly modulated between pre- and post-timepoint when the  $\chi^2$ -test statistic exceeded the significance level of the  $\chi^2$ -test statistic. None of the  $\chi^2$ -test statistics exceeded the significance level. A dependent T-test showed no significant differences between the means of  $\gamma$ -band CMC at pre- and post-timepoint as well. In this case,  $\gamma$ -band CMC was obtained by averaging CMC in the frequency range of either 30-40 or 30-60 Hz. In the grand average, the  $\gamma$ -band CMC in the unstable board condition was higher compared to the stable board condition, because  $\gamma$ -band CMC of the stable board was not significant while the  $\gamma$ -band CMC of the unstable board was. However, this was not confirmed by a significant difference between the two conditions according the difference in coherence test.



#### A. Left tibialis anterior

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# C. Right tibialis anterior

#### D. Right peroneus longus



**Figure 7.** Grand averages across participants and trials of the extended difference in coherence test based on the cortico-muscular coherence (CMC) estimates between the pre- and post-timepoint. Note that it cannot solely be deducted from the extended difference in coherence test plots how CMC changed as a function of the balance-training program and consequently indicate a significant CMC-increase or decrease. However, this can be determined from the CMC-estimates of pre- and post-timepoint. Horizontal green lines at 3.84 correspond to 95% confidence interval of  $\chi^2$  (Rosenberg et al., 1989).

Grand averages of the logarithmically transformed PSD estimates of the EEG channels Cz and CPz and rectified EMG of the shank muscles (right and left tibialis anterior and peroneus longus) of pre- and post-timepoint are depicted in Figure 8. EEG PSD did not modulate post-training compared to pre-training. EMG PSD was higher at the shank muscles of the stance leg compared to the non-stance raised leg. Furthermore, EMG PSD showed a decrease in the shank muscles of the stance leg across the complete frequency range at the post-timepoint compared to the pre-timepoint. This decrease was also observed in the other shank muscles (soleus and gastrocnemius medialis).



# A. Left stable









**Figure 8.** Grand average across trials and participants of logarithmically transformed power spectral density estimates of the EEG channels Cz and CPz and the tibialis anterior and peroneus longus estimates according the magnitude of the Hilbert transform. Rows correspond to different conditions. Pre-timepoint is marked in light-blue and post-timepoint in dark-red. Coloured shaded surfaces represent 1 standard deviation. PSD: power spectral density.

# 3.2.3 Level of muscle fatigue

Since the duration of experimental protocol was 4 hours which may have physically been demanding for the participants, fatigue at a muscular level might have occurred during the course of the protocol. Generally, the occurrence of muscle fatigue results in changes of amplitude- and phase-based measures of the surface EMG, or more precisely, the mean EMG amplitude increases while the median frequency of the EMG decreases (Lippold et al., 1960). Mean EMG amplitude and median frequency of the shank muscles of the stance leg contributing to significant CMC (see Figure 6) were estimated for each condition and timepoint. Means across pooled trials and participants in every condition were statistically compared using a dependent T-test with *Timepoint* as factor. This dependent T-test yielded no significant CMC (p > .05). Furthermore, the mean peak amplitude did not increase significantly in any of the muscles. Figure 9 illustrates the grand average across trials and participants of EMG amplitude and median frequency of the right and left tibialis anterior in the right- and left-legged conditions, respectively, as a typical example that there were no significant changes due to muscle fatigue.



**Figure 9.** Grand average across trials and participants of the **A**: median frequency and **B**: mean peak amplitude of the left tibialis anterior in the 'Left stable' and 'Left unstable' and the right tibialis anterior in the 'Right stable' and 'Right unstable' conditions.

# 3.2.4 Event-related results of the robot-controlled balance platform

Figure 10A shows a typical example of the perturbation time series on the robot-controlled balance platform. Considering the fact that the current study is the first study using the Haptic Master combined with the robot-controlled balance platform, the test-retest reliability of the balance platform was verified. Correlation coefficients of the imposed board angles were ranged from 0.981 to 0.998 between participants and from 0.983 to 0.997 between timepoints, implying that perturbation magnitude was equal among participants. The grand average of the rectified board angle of the robot-controlled balance platform at pre- and post-timepoint as a function of time is presented in Figure 10*B*. The board angle was positioned at the equilibrium angle of  $\pm 0^{\circ}$ . During every perturbation, the board angle approached its maximal value,  $|3^{\circ}|$ , after  $\pm 450$  ms followed by an exponential decay to the equilibrium angle of  $\pm 0^{\circ}$ , which is illustrated in the time window between -400 and 0 ms before the perturbation onset. When the board angle approached its equilibrium, the subsequent perturbation was imposed.



**Figure 10. A**: An example of the time series of the position-based perturbations as imposed during the robotcontrolled balance platform task. In the current study, the board angle of the balance platform has directly been manipulated instead of manipulating another mechanical quantity (e.g., moment of the board axis). The upper and lower plots illustrate the requested and actual perturbations time series, respectively. **B**: Grand average of the time-locked rectified board angle. Time = 0s corresponds to perturbation onset. Pre-timepoint is marked in light-blue and post-timepoint in dark-red. Shaded surfaces correspond to 1 standard deviation.

The left panels of Figure 11 depict the grand average time-locked data of channel Cz, the accelerometer at the top of the head, and the right and left rectified tibialis anterior as a result of the imposed perturbations on the robot-controlled balance platform. The potential changes at the channels of the sensorimotor cortex (Figure 11A) after perturbation onset may have been due to movement artefacts (e.g., the result of head movements). In order to exclude that these remarkable potentials between 200 and 400 ms after perturbation onset were artefacts, an accelerometer was attached at top of the EEG-cap between the channels Cz and CPz measuring the acceleration of the head in ML-direction (Figure 11B). The correlation coefficient between the time-locked activity of channel Cz and the ML-acceleration of the accelerometer of 9,900 perturbations (330 perturbations per time-point x 2 timepoints x 15 participants) yielded 0.05. Muscular responses (Figure 11C and D) can be observed in the right tibialis anterior did not change as a response to the perturbations. Note that the participant was consistently balancing on the right leg during this task.

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**Figure 11.** Grand averages of the time-locked data (e.g., ERP) and time-frequency relative power representations at **A**: channel Cz, **B**: medial-lateral acceleration of the head, **C**: rectified right tibialis anterior, and **D**: rectified left tibialis anterior averaged across 330 perturbations per timepoint and 15 participants. Time = 0s corresponds to perturbation onset. In the left panels, pre-timepoint is marked in light-blue and post-timepoint in dark-red. Shaded surfaces correspond to 1 standard deviation. Note that participants were balancing on their right leg in this condition.

The middle and right panels of Figure 11 depict the relative power TFRs of both pre- and post-timepoint. The relative power was obtained by dividing every TFR-element with the averaged power of -200 till 0 ms. The cortical sensorimotor channels revealed an upregulated  $\theta$ -band power and suppression of the high  $\beta$ -band (±25-30 Hz) and low  $\gamma$ -band (±30-35 Hz) power within the time window of 200 till 400 ms

(Figure 11A). Following the low correlation coefficient between the time-locked time series of the sensorimotor channels and accelerometer, the relative  $\theta$ -band power modulation cannot be observed in the TFR of the accelerometer either, revealing that the cortical ERPs were not due to movement artefacts (Figure 11B). The cortical sensorimotor channels showed a downregulation of relative  $\beta$ - and  $\gamma$ -band power within the time window of 200 till 400 ms after perturbation onset. The down-regulated high  $\beta$ -power in the sensorimotor channels likely reveal local event-related desynchronization preceding movement initiation, especially, since increased  $\theta$ -band activity in the right tibialis anterior was observed after 450 ms (Figure 11*C*). The relative  $\theta$ -band power increased as observed in the right tibialis anterior (stance leg) corresponds to the ERP changes of the right tibialis anterior (raised leg) (Figure 11*D*) as the ERP of the left tibialis anterior did not change either. The other pairs of homologous shank muscles (e.g., peroneus, soleus, and gastrocnemius medialis) showed comparable findings regarding the ERP- and relative power TFR-courses. In addition, ERPs and relative power TFRs also presented equivalent characteristics in terms of the modulation timing of the frequency content.

Figure 12 illustrates the grand average across trials and participants of the cortico-muscular timefrequency coherence representations between the mid-central EEG channel Cz and the shank muscles (tibialis anterior, peroneus longus, gastrocnemius medialis, and soleus) at both timepoints and on both legs. Channel Cz was selected as topological EEG plots revealed (most) significant CMC around this channel for the four shank muscles. The TFRs show  $\gamma$ -band CMC centred around the 40 Hz in the stance leg (Figure 12A). This increased  $\gamma$ -band CMC was not present in the non-stance leg (Figure 12B), which was also observed in the stable and unstable balance conditions. The  $\gamma$ -band CMC-magnitude in the stance leg at the post-timepoint was lower, which is also visible in the TFRs in which CMC at posttimepoint is subtracted of CMC at pre-timepoint (Figure 12E). CMC-magnitude of the non-stance leg at the post-timepoint was comparable to the pre-timepoint (Figure 12D) and CMC was not modulated between pre- and post-timepoint (Figure 12F). CMC-modulation in both legs was statistically evaluated using cluster-based permutation tests with a dependent T-test. Resulting significant T-statistics of both stance and non-stance leg are visualised in Figure 12G and H, supporting the expectation that  $\gamma$ -band CMC in the stance leg was significantly lower at the post-timepoint than at pre-timepoint.  $\gamma$ -band CMCmodulation in the non-stance leg was not significantly different.







**Figure 12.** Grand averages across trials and participants of the cortico-muscular time-frequency coherence representations between the mid-central EEG channel Cz and the average of four shank muscles (tibialis anterior, peroneus longus, gastrocnemius medialis, and soleus) at the pre-timepoint on the **A**: right and **B**: left leg and post-timepoint on the **C**: right and **D**: left leg. **E-F**: Grand average of the difference in cortico-muscular time-frequency coherence presentations between pre- and post-timepoint. CMC at post-timepoint was subtracted from CMC at pre-timepoint. **G-H**: (Significant) T-values of the CMC-modulation between the pre- and post-timepoint for the right and left leg. Significant T-values are opaque.

# 3.3 Correlative analysis of neurophysiological and behavioural adaptations

The mean COP- and COM-velocity on the stable and unstable board were selected for this correlative analysis as behavioural variable. CMC of the tibialis anterior (TA) and peroneus longus (PN) was converted into a scalar value by averaging  $\gamma$ -band CMC (35-40 Hz) and pooling five trials of each timepoint. Figure 13 reveals the scatterplots with Pearson's correlation coefficients and significance level of the mean COP-velocity and CMC(-modulation) (Figure 13A) and the mean COP-velocity and CMC(-modulation) (Figure 13B) of the 20 participants. Significant Pearson's correlation coefficients identified a weak and weak-to-moderate positive relationship between mean COM-velocity and  $\gamma$ -band CMC as estimated with the tibialis anterior at pre- and post-timepoint, respectively. None of the Pearson's correlation coefficients were not significant between CMC-modulation and behavioural changes as a result of the balance-training program (right panels). Furthermore, no significant correlation coefficients were observed between mean COP-velocity and CMC. In the robotic-controlled balance platform, none of the Pearson correlation coefficients showed a significant correlation between CMC and mean COM-velocity and CMC and RMS of the head acceleration. To estimate correlation coefficients, perturbations and trials were pooled for participants and timepoints.



**Figure 13. A:** Scatterplots including least-squares line (green lines) and Pearson's correlation coefficients (r) between **A:** the mean COP-velocity and CMC(-changes) and **B:** the mean COM-velocity and CMC(-changes) as a function of the balance training program. Cz: EEG channel Cz; TA: tibialis anterior; PN: peroneus longus.

В.

Α.



#### 4 Discussion

The efficacy of learning to balance unilaterally for a short period has been shown at a behavioural level though the neuro-physiological mechanisms underlying the improvements are currently not well understood. Cortico-muscular oscillations in the  $\gamma$ -band (30-60 Hz) can play a pivotal role during dynamical tasks (e.g., postural control). Here, we specifically targeted how  $\gamma$ -band cortico-muscular coherence (CMC) changes as a result of balance-training. It was hypothesized that  $\gamma$ -band CMC modulates during this period of short-term motor learning and that these changes in  $\gamma$ -band CMC correlate with changes at the behavioural level. The main findings are that significant  $\gamma$ -band CMC with a peak around 40 Hz was found in the stable, unstable, and robot balance tasks at pre- and post-timepoint.  $\gamma$ -band CMC was not consistently modulated across the balance-training program among the three balance tasks. While the perturbation-induced  $\gamma$ -band CMC decreased, the continuous  $\gamma$ -band CMC did not change as a result of short-term motor learning. As hypothesized, the mean COP-velocity was significantly across the balance-training program. Furthermore, the decrease in COM-velocity was significantly higher when participants balanced on the unstable board than on the stable board. Finally, there were weak-to-moderate positive relationships between the  $\gamma$ -band CMC and mean COM-velocity.

# 4.1 Cortico-muscular γ-band oscillations in motor control

Previous studies have described the existence of  $\gamma$ -band CMC related to motor control, specifically dynamical tasks (Mehrkanoon et al., 2014; Mendez-Balbuena et al., 2011; Omlor et al., 2007; Petersen et al., 2012).  $\gamma$ -band CMC as currently presented with a peak around 40 Hz likely corresponds to the Piper rhythm (Brown et al., 1998). The Piper rhythm describes the tendency of cortico-spinal neural circuits to fire phase-locked at a frequency around 40 Hz in strong isometric and slow-dynamical tasks (Brown, 2000). This cortico-spinal communication may occur from the cortical areas along fast pyramidal tracts (e.g., mono-synaptic neural pathways) to the motor-pool units (Lemon, 2008). Although the Piper rhythm has rarely been observed in human motor control, the here-reported significant  $\gamma$ -band CMC may be ascribed to the Piper rhythm, since it peaked between 35 and 41 Hz (Salenius et al., 1996). Furthermore, the Piper rhythm may originate in the cortex from the midline of the sensorimotor cortex in dynamical lower leg contractions (Salenius et al., 1997) as shown by the CMC-estimates between the sensor-level EEG (channel Cz) and rectified EMG of the shank muscles (tibialis anterior and peroneus) in the current study. It is unlikely that the significant  $\gamma$ -band CMC(-modulation) was the result of muscular fatigue, since the mean peak amplitude and median frequency of the EMG signals did not increase and decrease, respectively, between pre- and post-timepoint (Lippold et al., 1960).

Significant  $\gamma$ -band CMC was only found in the shank muscles of the stance leg in both continuous and perturbation-induced balance tasks, while significant  $\gamma$ -band CMC in the non-stance, raised leg was lacking. The upregulation in the cortico-spinal drive was purely related to the increased unilateral synchronized activity between the unilateral sensorimotor cortex (e.g., the M1-cortex) and the contralateral shank muscles of the stance leg. The ipsi-lateral synchronized activity from one hemisphere to the shank muscles of the stance leg was not present. The same held for this hemisphere and the shank muscles of the non-stance leg. Significant cortico-muscular  $\gamma$ -band oscillations in the non-stance, raised leg may not be present because raising and holding one leg off the ground mainly leads to static isometric contractions of the shank and thigh muscles in this leg (Torres-Oviedo and Ting, 2010). These



isometric contractions possibly limit the generation of cortico-muscular  $\gamma$ -band oscillations as the existence of this type of oscillations is more profoundly expressed in dynamical tasks (i.e. non-isometric tasks) (Mendez-Balbuena et al., 2011). On the other hand, one could expect that the isometrically contracting muscles in the non-stance leg may trigger cortico-muscular  $\beta$ -oscillations as these have been associated with the maintenance of a certain sensorimotor state (Mehrkanoon et al., 2014). However, other studies show that no  $\beta$ -oscillations can be observed below a certain force threshold (Gross et al., 2005; Perez et al., 2012), suggesting that the isometric contractions as made in the non-stance leg were simply too weak to generate  $\beta$ -oscillations between the sensorimotor cortex and shank muscles of the non-stance leg. Furthermore, no 'precision' was required to raise the leg as this generates corticomuscular  $\beta$ -oscillations as well (Kristeva et al., 2007). The distinguished function of the stance and nonstance leg is in line with the behavioural findings of another study, which reported a positive and negative covariance for the stance and non-stance leg, respectively, relative to the other segments of the body (van Dieën et al., 2015). While the occurrence of  $\gamma$ -band CMC with muscles of the lower extremities depends on the leg, CMC-estimates based on more proximal muscles (e.g., external oblique, gluteus medius and iliocostalis) tended to show bilateral rather than unilateral  $\gamma$ -band CMC. These muscles have been associated with the dynamical sway rather than static contractions during postural control (Klous et al., 2011). The presence of  $\gamma$ -band CMC estimated with the proximal muscles and stance-leg muscles, and the absence in the muscles of the non-stance leg is also consistent with the difference in positive and negative covariance in the COM-path for the stance and non-stance leg relative to the other parts of the body. Besides the  $\gamma$ -band CMC, significant  $\theta$ -band CMC was observed between 4-8 Hz in the continuous stable and unstable board tasks.  $\theta$ -band CMC has been associated with attention (modulation) during the transient motor learning phase in precision tasks (Hori et al., 2013). Despite the fact that several studies indicated a phase-amplitude coupling between the  $\theta$ - and  $\gamma$ -band in the cortical regions (Canolty et al., 2006; Fries, 2009), this coupling has not been observed as corticalmuscular coupling in human motor control. Speculatively, the significant  $\theta$ -band CMC might reflect attention, since the unilateral balance tasks were quite demanding, however, we cannot exclude that these oscillations emerged from movements artefacts, for example, physiological tremors (Raethjen et al., 2002).

Although significant  $\gamma$ -band CMC was found, we attempted to improve the CMC-estimates by dimensionality reduction of the EMG through multivariate analysis. The main reason to decompose the EMG time series was to increase the signal-to-noise ratio of the CMC-estimates. Multivariate decomposition of the EMG time series was performed with non-negative matrix factorization (NNMF) (Lee and Seung, 2001) (results have not been presented in this thesis), as the EMG was rectified and NNMF is constrained to only have non-negative values (Cheung et al., 2005). The results of the current study, however, showed that the decomposition of EMG time series led to a loss of the amplitude- or phase-based variance that was responsible for the characteristic  $\gamma$ -band CMC peak as observed in CMC-estimates with the non-decomposed EMG. This may be the result of the fact that the first modes of NNMF only include the variance that was associated with bursts of EMG activity, and, therefore, a high amplitude. These bursts of EMG activity are quite common in unilateral balance tasks (van Dieën et al., 2015). Coherence may significantly be affected by these bursts of EMG activity (Bortel and Sovka, 2006) as they largely contribute to the amplitude-based (i.e. power) normalisation of the coherence (Conway et al., 1995). Likewise, the estimation of CMC between EEG and NNMF-modes that merely consists of EMG bursts will result in lower coherence values.

# 4.2 Cortico-muscular γ-band oscillations in motor learning

Time-locked  $\gamma$ -band CMC in the perturbation-induced balance task was significantly decreased in the stance leg across the balance-training. This  $\gamma$ -band decrease was observed from -0.4s before till 0.6s after the mechanical perturbation. The fact that  $\gamma$ -band CMC is constantly modulated rather than event-related (e.g., the dynamical phases of mechanical perturbations) may be the consequence of the rapid imposition of consecutive perturbations (±0.8 Hz) and the short time periods at the equilibrium position (±100 ms) between consecutive perturbations. To be able to find an event-related  $\gamma$ -band CMC response to the perturbations and relate the  $\gamma$ -band CMC to different phases of the perturbations a longer period in the equilibrium position is required.

The observed  $\gamma$ -band CMC decrease compromises a lower phase-locked synchronization in the corticospinal drive as a result of short-term balance learning. Despite the decline in the  $\gamma$ -band CMC in the perturbation-induced balance task, no time-locked y-band power-modulation was observed in the sensorimotor cortex and shank muscles. The decline in  $\gamma$ -band CMC with no power-modulation suggests a phased-based modulation with amplitude-constancy of these neural oscillations. Similar findings of amplitude-independency in cortico-spinal connectivity estimates have been found in static response tasks by contrasting coherence and phase synchronization for  $\beta$ -oscillations (Van Wijk et al., 2009). However, the interpretation of the cortico-muscular  $\gamma$ -oscillations is different from the  $\beta$ -oscillations (Kopell et al., 2000). It has been shown that  $\gamma$ -band CMC was associated with corrections to sensory prediction errors (Schoffelen et al., 2005). Mehrkanoon and co-workers (2014) found a higher dual-band CMC in the  $\alpha$ and  $\gamma$ -band after trials in which a compensation was required to correct for manual force overshoot. As proposed by these authors, the  $\alpha$ -band CMC may originate from the visual feedback that was given to the participants in that study. Other studies consisting of paradigm including a dynamical task and visual feedback also observed dual-band coherence (Buffalo et al., 2011; Van Wijk et al., 2009). Since no visual feedback was provided to the participants in the current study this may explain why no  $\alpha$ -band CMC was observed.

We were not able to show that the perturbation-induced  $\gamma$ -band CMC was associated with movement corrections as the time-locked excursion in the ML-direction of the right shank markers (heel, knee, and hip) did not correspond to the time-locked  $\gamma$ -band CMC-changes. Furthermore, no significant Pearson's correlation coefficients between  $\gamma$ -band CMC and behavioural data were observed, suggesting that the decrease in  $\gamma$ -band CMC was not associated with a change at a behavioural level. This lack of significant correlations may partially be the result of the inter-individual differences as found in both  $\gamma$ -band CMC and mean COP- and COM-velocity. Inter-individual differences at behavioural level were found in the initial unilateral balance performance and response to the balance-training program. However, the significant Pearson's correlation coefficients only identified weak-to-moderate relationships between  $\gamma$ band CMC and mean COP- and COM-velocity yielding that inter-individual differences at a neurophysiological level did not correspond to the inter-individual differences at a behavioural level. Previous studies found strong positive correlations between cortico-muscular oscillations and static task performance (Ushiyama et al., 2017). However, these studies examined the cortico-spinal synchronization in the  $\beta$ -band. The modulation of  $\gamma$ -band CMC in relation to short-term motor learning seems to be different from  $\beta$ -band CMC-modulation as cortico-spinal  $\beta$ -band oscillations are usually upregulated after motor learning and are correlated to improvements at behavioural level as well (Larsen et al., 2016; Mendez-Balbuena et al., 2011; Perez et al., 2006). This suggests a different functional role for  $\beta$ - and  $\gamma$ -band CMC during motor learning as found in a modelling study as well (Kopell et al., 2000).

The upregulation of  $\beta$ -band CMC has directly been associated with an increased neural firing behaviour via mono-synaptic loops along the cortico-spinal tract (Perez et al., 2006) as a result of reorganization of the cortical representations (Conway et al., 1995). On the contrary,  $\gamma$ -band CMC-modulation is most likely associated with a lower cortico-spinal excitability describing a lower firing rate along fast pyramidal pathways due to motor learning.

While the perturbation-induced  $\gamma$ -band CMC decreased, the continuous  $\gamma$ -band CMC did not change after balance-training. A decrease was found in the mean COP-velocity suggesting modulation of neural structures other than the cortical areas. The involvement of sub-cortical neural structures (e.g., brain stem and spinal level) in postural control has already been shown based on the latency of reactions to postural disturbances (Jacobs and Horak, 2007; Taube et al., 2006). The sub-cortical and spinal circuits adapt to changing requirements during balance training by adjusting reflex behaviour (Taube et al., 2008). Future research may address the modulation of intermuscular coherence as it is believed that this type reflects the synchronization level at sub-cortical and spinal levels (Boonstra et al., 2009).

The different findings in  $\gamma$ -band CMC-modulation between the continuous and perturbation-induced tasks are likely the result of task differences. While the perturbation-induced task was related to the anticipation to mechanical perturbations, the continuous tasks did not require anticipatory behaviour. The first task requires repetitive reactive compensation in order to maintain balance after the perturbations, whereas the latter does not. Since the mechanical perturbations had a consistent magnitude of  $+3^{\circ}$  or  $-3^{\circ}$  and were imposed at a rate of  $\pm 0.8$  times per second, the variability between perturbations merely was the variable time of  $\pm 100$  ms between perturbations and the direction, either clockwise or counter clockwise. Consequently, the predictability of the perturbations was high. This high predictability may partially be associated with the lower  $\gamma$ -band CMC, because a higher CMC in the  $\gamma$ band may be the result of an increased probability on sensory prediction errors (Schoffelen et al., 2011). As participants knew what to expect when they were balancing unilaterally on the robot-controlled balance platform for the second time (e.g., at post-timepoint), there may have been fewer sensory prediction errors, for example, a decreased proprioceptive feedback (Dietz et al., 1992). The lower inconsistency between the expected and real balance state would then lead to a decrease in  $\gamma$ -band CMC. On the contrary, the continuous tasks contained a lower predictability as participants could balance freely and were not constrained to anticipate on mechanical perturbations. This lower predictability may result in an equal probability in sensory prediction errors at pre- and post-timepoint and hence no modulation of  $\gamma$ -band CMC.

# 4.3 Behavioural results

At the behavioural level, mean COP-velocity decreased in the continuous tasks significantly across the balance-training program. No significant main effect of *Timepoint* was found in case mean COM-velocity was the predictor variable. However, a significant two-way interaction of *Timepoint* x Board was found, meaning that the decrease of mean COM-velocity was higher for the unstable board than for the stable board. The behavioural modulation across a short-term balance-training program is in line with findings by van Dieën et al. (2015), who found a decrease in COM-path (mean COM-velocity times duration of the trial) across 30 minutes of unilateral postural training. However, the current study was not able to show a significant decrease in mean COM-velocity. The mean COM-velocity as estimated might be an underestimation of the mean COM-velocity based on full-body kinematics (van Dieën et al., 2015). Here,

the mean COM-velocity was estimated between the left and right SIPS-markers, which can be considered as a rough estimate of the COM-sway. However, possible movements of the lower and upper limbs which highly contribute to the maintenance of balance are neglected, (Horak and Nashner, 1986). Van Dieën and colleagues found a significant decrease in COM-path using 17 markers to estimate the COM. One may expect that if the COM had been estimated based on the full-body kinematics represents the actual COM more accurately and gives a higher difference in mean COM-velocity between pre- and posttimepoint.

The mean COM-velocity and RMS of the head acceleration in the perturbation-induced task did not change across the balance-training program, which was not expected based on the decrease in  $\gamma$ -band CMC. This discrepancy between the neurophysiological and behavioural level may be due to the fact that the mean COM-velocity between the right and left SIPS and RMS of the head acceleration do not operationally represent the change of  $\gamma$ -band CMC between the sensorimotor cortex and shank muscles.

#### 4.4 Future research

Future steps may consist of a beamforming procedure that considers both amplitude- and phase-based modulation between the cortex and muscles, for example, the estimation of the spatial filters based on maximal CMC or maximal CMC-modulation across the balance-training program. De Vries et al. (2016) searched cortical areas that show maximal CMC, decomposed the corresponding virtual source signals with singular value decomposition and found significant  $\beta$ -band CMC in a bimanual task. In this thesis, the spatial filters for the beamforming procedure were based on the  $\gamma$ -band power modulation of all EEG channels, which is only based on amplitude modulation. Using the consecutive virtual source signals of each hemisphere to estimate CMC with the contralateral muscle activity abolished the significant  $\gamma$ -band CMC around 40 Hz (Appendix C) and yielded no significant  $\gamma$ -band CMC-modulation as a result of balance training. Therefore, spatial filters that are directly related to the cross-spectral interactions between EEG and EMG (e.g., CMC) may improve the source localisation and the estimation of virtual source signals. However, the computation of the DICS-beamformers that optimize CMC was beyond the scope of this thesis and will be addressed in future research.

No significant correlations were present between the changes of  $\gamma$ -band CMC and mean COP- and COMvelocity, hampering the interpretation of CMC-modulation. Also, this might indicate that significant  $\gamma$ band CMC does not reflect a crucial aspect of motor learning. Future work might clarify the interpretation of the CMC-modulation by addressing CMC-directionality and questions whether both sensory- and motor-related pathways are equally contributing pre- and post-training. Coherence (e.g., normalized cross-spectral density) is inadequate to estimate directionality, which is a generally known disadvantage of using linear correlations (Zalesky et al., 2012). The directionality of CMC has been reviewed by Baker (2007) who showed the occurrence of CMC in both feedback and feedforward neural pathways in motor control (Baker, 2007). During motor learning in postural control, however, re-weighting of sensory ascending and motor descending signals occurs compromising to the specific requirements to maintain balance (Sober and Sabes, 2003). For example, the use of different sensory input types (i.e. visual, vestibular, and proprioceptive) is up- or down-regulated during the short-term learning period of a novel unilateral balance tasks (Davis et al., 2011). In the initial learning phase, proprioceptive inputs are essential to control unilateral balance while the importance of the visual and vestibular feedback increases across the learning period (van Dieën et al., 2015). It should be mentioned that here the contribution of visual feedback may be limited as participants were constrained to look at a white cross on the wall to reduce EEG artefacts. For the results of the present study, it could have been expected that CMC-directionality may modulate, since local muscular synchronization (e.g., power) changed, reflected by a decreased PSD of the EMG. However, it should be taken into account that the decrease of PSD of the EMG my also reflect a lower recruitment of sub-cortical or spinal levels. To investigate the CMC-directionality, the cortico-muscular phase-locked causality should be estimated, which can be determined by using the phase-slope index (Nolte et al., 2008) or partial directed coherence (Schnitzler and Gross, 2005). The application of the phase-slope index and partial directed coherence to the current data may enhance the value of the results as found in this thesis and renders the interpretation that  $\gamma$ band CMC is involved in full-body dynamical tasks as challenging postural control tasks.

Finally, the phase-locked synchronization between neural populations is not merely limited to corticomuscular interactions but can also be extended to cortical populations in different brain areas (e.g., cortico-cortical coherence) and different motor-unit pools (e.g., intermuscular coherence). Although cross-spectral coupling between these neural structures has been investigated in paradigms including CMC during motor learning (e.g., Hori et al., 2013; Pizzamiglio et al., 2017), the phase-locked modulation in relation to short-term learning is poorly understood. The extension of the current study with both EEG-EEG and EMG-EMG coherence may clarify whether the phase-locked modulation is limited to the cortical level or whether changes occur at the sub-cortical and spinal level as well (Boonstra et al., 2009). The decrease in  $\gamma$ -band CMC in the perturbation-induced task may be accompanied by increases in inter-muscular coherence at lower frequencies suggesting higher synchronization between sub-cortical and spinal structures or between different motor unit pools (Danna-Dos-Santos et al., 2014). The extension with EEG-EEG and EMG-EMG coherence is especially interesting, since the current study shows behavioural improvements across the short-term learning period while the CMC on its own seems not sufficient to explain these behavioural improvements.



# 5 Conclusions

The results of the present study highlight the existence of cortico-muscular  $\gamma$ -band oscillations in novel unilateral surface stability tasks. These cortico-muscular  $\gamma$ -band oscillations correspond to the Piper rhythm. Perturbation-induced  $\gamma$ -band cortico-muscular coherence responses as a result of the short-term balance-training program decreased while the continuous  $\gamma$ -band cortico-muscular coherence did not decrease suggesting task-dependent phase-locked modulation during unilateral balance tasks. The decrease  $\gamma$ -band cortico-muscular coherence in the perturbation-induced task might be the result of the rhythmic occurrence of consecutive perturbations. The lack of the significant  $\gamma$ -band cortico-muscular phase-locked modulation in the continuous tasks suggests that the behavioural balance improvements (e.g., lower media-lateral postural sway) were not the result of changes in neural oscillations as derived from cortical areas. Nevertheless, the present results support cortical involvement in sensorimotor control during full-body surface stability tasks, because of the significant  $\gamma$ -band cortico-muscular coherence.

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# **Appendix A**

The aim of these pilots of Appendix A was to investigate whether unilateral balance tasks actually result in a synchronized activity between cortical regions and shank muscles. During this particular pilot study, the participant had to perform unilateral surface stability tasks on the same balance board as used for the current study. Simultaneously, 64-channel EEG was recorded with surface EMG activity of four shank muscles: the left and right tibialis anterior (TA) and peroneus longus (PN). However, there was no balance-training program included in the protocol. The results of this pilot study showed CMC around 40 Hz between the averaged EEG signals of the sensorimotor cortex (C1-C6, FC3-FC4, and CP3-CP4) and the rectified surface EMG signals of the TA and PN in the stance leg, while this this CMC peak is not present in the non-stance raised leg. Figure 14A illustrates a unilateral condition at the left leg and the Figure 14B above was a unilateral condition at the right leg. The obtained CMC peak around 30-60 Hz is consistent with studies that investigated other dynamical tasks as walking and force output generation, and is called the Piper rhythm, which has been associated with dynamical tasks. The results of this pilot study highlighted the existence of cortico-muscular coherence (CMC) within the  $\gamma$ -frequency band (30-60 Hz).



**Figure 14.** Individual coherence spectra between averaged EEG signals overlying the motor cortex (C1-C6, FC3-FC4, and CP3-CP4) and the rectified EMG signals of the tibialis anterior and peroneus of the dominant leg.  $TA_R$ : right tibialis anterior,  $PE_R$ : right peroneus,  $TA_L$ : left tibialis anterior,  $PE_L$ : left peroneus. **A:** the dominant leg is the left leg (lower left graph). **B:** the dominant leg is the right leg (upper right graph).



# Appendix **B**

Since the robot-controlled balance platform had not been used before, a pilot study was performed to investigate the cortical responses when consecutive perturbations were imposed. Therefore, the aim of this pilot study was two-fold: (1) to investigate how many trials should be performed perturbations should be imposed in order to get an accurate estimate of the ERP and (2) to investigate the response of the sensorimotor cortex. During this pilot, the participant performed 10 trials in which 38 perturbations were imposed in each trial. The participant was asked to anticipate adequately on the imposed perturbations and to stabilize the COM to the pre-perturbation position. 64-channel EEG was recorded when perturbations were imposed. Besides to the standard EEG-cleaning as described in paragraph 2.4 (see Methods – Data analysis), EEG time series were time-locked from 0.40 s before and 0.60 s after the perturbation and pooled within a trial. Time-locked evoked potentials of pooled perturbations within a trial and the grand average are illustrated in Figure 15.



**Figure 15.** Individual time-locked EEG response at the CPz-channel as obtained during the pilot. Onset represents the start of the perturbation imposition. The upper panel illustrates the pooled perturbations of 10 trials that all consists of 38 perturbations. Shaded surface corresponds to 1 standard deviation. Note that the limits of the y-axis of both panels are different. The lower panel corresponds to the grand average of the 380 perturbations (10 trial x 38 perturbations). Shaded surface corresponds to 1 standard error of the mean.

EEG time series of the motor cortex were transferred to the frequency domain according a multitaper convolution method with a Hanning-taper as implemented in FieldTrip. Consequently, time-frequency representations (e.g., time-frequency power plots) were constructed. Time-frequency power plots of the sensorimotor cortex show frequency power modulation of the  $\theta$ - and  $\delta$ -frequency band (0.5-4, and 4-8 Hz, respectively) (Figure 16). This modulation of the  $\delta$  and  $\theta$  band corresponds to the N200- and P300-peak of Figure 15.



**Figure 16.** Grand average time-frequency representation (TFR) at the CPz-channel. TFR describes the average of 380 perturbations: 10 trials each consisting of 38 perturbations. This TFR represents the absolute power (in dB) as observed at channel CPz with a baseline between -0.3 and -0.1s. Time = 0s indicates the perturbation onset as imposed by the Haptic Master. Note that the EEG data has been bandpass filter between 2 and 30 Hz before construction of the TFR.

To determine the number of perturbations after this pilot to include in the experimental protocol a bootstrapping method was applied. 95% *CI* was estimated based on the random selection of the time-locked potential at Cz of 2-380 perturbations. This estimation procedure of the random selection of potentials was repeated for 5,000 times. The resulting exponential decay showed that the imposition of 320 perturbations would lead to an increase of <5% of the 95% *CI* [-0.250; 0.287  $\mu$ V] compared to the estimated 95% *CI* of the grand average ERP based on 380 perturbations [-0.240; 0.276  $\mu$ V].



5

# Appendix C

#### Reconstruction of source signals

This appendix contains the results of the DICS-beamforming procedure. Figure 17 shows orthogonal anatomical maps as a result of the DICS-beamforming. Recall that this DICS-beamforming procedure was based on searching for the cortical area revealing the (most) significant  $\gamma$ -power modulation between pre- and post-timepoint. The MNI-coordinates of these focal cortical sources for right and left hemisphere were equal to [3.0; 8.0; 71.0] cm and [-8.0; 11.0; 64.0] cm, respectively. MNI-coordinates are located in the bilateral Brodmann's area 6 and correspond to the right and left supplementary motor areas. Robustness of the significant cortical sources was checked by repeating the cluster-based permutation test 20 times for 19 (out of 20) participants excluding one participant each iteration. MNI-coordinates were converted to CTF-coordinates to obtain the corresponding spatial filters. To extract the virtual source signals, spatial filters were matrix multiplied with channel-level EEG. These virtual source signals that explains the most variance. Consequently, the right and left hemispheric virtual source signals as found with singular value decomposition were used to estimate CMC with the contralateral rectified EMG.







**Figure 17.** Grand average of the orthogonal anatomical maps of the **A**: right and **B**: left hemisphere. Highlighted small yellow-coloured areas mark significant T-values containing the cortical sources where maximal  $\gamma$ -power modulation was detected between pre- and post-timepoint. DICS-beamformers were frequency locked in the frequency band of 30-60 Hz.

The CMC-estimates between the 'beamformed EEG' and the rectified EMG signals of the shank muscles highlighted two important phenomena (Figure 18). Firstly, the  $\gamma$ -band CMC-peak around 40 Hz disappeared, which has been observed in the CMC-estimates based on the sensor-level EEG time series. Furthermore, no modulation of  $\gamma$ -band CMC between pre- and post-timepoint was observed according the difference in coherence test. As mentioned in *Discussion*, future research will estimate the spatial filters of the DICS-beamformers based on the  $\gamma$ -band CMC-modulation across the balance-training program directly instead of the cortical  $\gamma$ -power modulation as used in the current analysis.



**Figure 18.** Grand average of CMC-estimates between 'beamformed' EEG and right and left tibialis anterior in the right and left unstable condition, respectively. DICS-beamforming was based on the identification of the anatomical sources contributing significantly to cortical  $\gamma$ -power modulation across the balance-training program.