Unilateral transfemoral amputees exhibit altered strength and dynamics of muscular co-activation modulated by visual feedback

T Krauskopf, T B Lauck, L Klein, M Beusterien, M Mueller, V Von Tscharner, C Mehring, G W Herget, T Stieglitz, and C Pasluosta

1 Laboratory for Biomedical Microtechnology, Department of Microsystems Engineering, University of Freiburg, Freiburg im Breisgau, Germany
2 BrainLinks-BrainTools Center of Excellence, University of Freiburg, Freiburg, Germany
3 Department of Orthopaedics and Trauma Surgery, University Medical Center, Freiburg im Breisgau, Germany
4 Sanitätshaus Pfänder, Freiburg im Breisgau, Germany
5 Human Performance Laboratory, University of Calgary, Calgary, Canada
6 Bernstein Center Freiburg, University of Freiburg, Freiburg, Germany
7 Faculty of Biology, University of Freiburg, Freiburg im Breisgau, Germany
8 Equal senior contribution.

* Author to whom any correspondence should be addressed.
E-mail: cristian.pasluosta@imtek.uni-freiburg.de

Keywords: neuromuscular control, amputee, intermuscular coupling, dynamics, wavelet coherence, balance, sensory feedback

Abstract

Objective. Somatosensory perception is disrupted in patients with a lower limb amputation. This increases the difficulty to maintain balance and leads to the development of neuromuscular adjustments. We investigated how these adjustments are reflected in the co-activation of lower body muscles and are modulated by visual feedback. Approach. We measured electromyography (EMG) signals of muscles from the trunk (erector spinae and obliquus external), and the lower intact/dominant leg (tibialis anterior and medial gastrocnemius) in 11 unilateral transfemoral amputees and 11 age-matched able-bodied controls during 30 s of upright standing with and without visual feedback. Muscle synergies involved in balance control were investigated using wavelet coherence analysis. We focused on seven frequencies grouped in three frequency bands, a low-frequency band (7.56 and 19.86 Hz) representing more sub-cortical and spinal inputs to the muscles, a mid-frequency band (38.26 and 62.63 Hz) representing more cortical inputs, and a high-frequency band (92.90, 129 and 170.90 Hz) associated with synchronizing motor unit action potentials. Further, the dynamics of changes in intermuscular coupling over time were quantified using the Entropic Half-Life. Main results. Amputees exhibited lower coherency values when vision was removed at 7.56 Hz for the muscle pair of the lower leg. At this frequency, the coherency values of the amputee group also differed from controls for the eyes closed condition. Controls and amputees exhibited opposite coherent behaviors with visual feedback at 7.56 Hz. For the eyes open condition at 129 Hz, the coherency values of amputees and controls differed for the muscle pair of the trunk, and at 170.90 Hz for the muscle pair of the lower leg. Amputees exhibited different dynamics of muscle co-activation at the low frequency band when vision was available. Significance. Altogether, these findings point to the development of neuromuscular adaptations reflected in the strength and dynamics of muscular co-activation.

1. Introduction

The permanent flow of somatosensory ( proprioceptive and cutaneous), vestibular, and visual information to the central nervous system (CNS) is essential for maintaining postural balance and stable gait [1]. As upright standing is a non-static and dynamic task, it requires the postural control system to constantly use the incoming sensory information to adjust muscle activity [2].
A small but universal set of motor programs are used to maintain balance and to control posture [3]. These balance strategies include the ankle-strategy, where the body moves in all directions around the ankle using lower leg muscles [4], and the hip-strategy that involves a rotation of the body around the hip utilizing muscles from the trunk and hip [5]. Both strategies are combined to keep an upright stance [6, 7]. The neural control of these motor programs becomes more efficient and coordinated by the use of functional muscle synergies, which are neural strategies used to reduce the controlled degrees of freedom during the execution of a specific movement [8–11]. The formation of functional muscle synergies is sometimes referred to as muscle modes (M-modes), reflecting the hypothetical control variable of a virtual muscle, i.e. muscles that are involved in a specific movement and are synergistically activated by a common neural signal [12–14].

Mechanically, patients with a lower limb amputation exhibit a limited ability to make fine motor adjustments during upright standing because of a reduced size of their base of support and an increased joint stiffness [15]. For instance, the stability of amputees while standing on a compliant surface is impaired in the mediolateral direction, presenting up to four times higher postural sway than able-bodied individuals [16]. This is mainly caused by a reduction of ankle mobility, weakening the compensation for the greater movement of the body’s center of mass when standing on such surfaces [17].

Neurologically, patients with a lower limb amputation exhibit a reorganization of their postural control system due to incomplete sensory feedback [18]. The sensory information from the intact limb thus becomes of utmost importance for balance control in lower limb amputees. The remaining sensory inputs gain a stronger weight in the contribution to the overall sensory feedback required for postural balance control [19, 20]. Supplementary somatosensory information delivered via implantable electrical stimulation devices enhanced mobility and improved the ability to make fine adjustments to movements in lower limb amputees [21]. Restoring sensory feedback via intraneural stimulation also improved walking speed, lowered metabolic cost, and reduced phantom pain [22]. In transtibial amputees, sensory neuroprostheses improved stability during upright standing, especially when other sensory inputs were weakened [23].

In previous work, we observed that during quiet standing the dynamics of the adjustments of the center of pressure (CoP) of unilateral transfemoral amputees differ from able-bodied controls and that compensatory adjustments of the intact limb are required to counteract the loss of mechanical stability and sensory feedback from the amputated limb [24]. As the CoP adjustments are the final motor output of many control strategies of the central and peripheral nervous system acting on the muscular system, we sought to investigate intermuscular coupling in the lower body muscles in an attempt to explain the atypical dynamics of the CoP adjustments observed in this patient population.

Xu and colleagues compared in a recent study muscle forces coordination of the intact leg of transfemoral amputees and able-bodied controls during walking. Their findings point to the establishment of a control strategy for meeting specific joint moment requirements during gait [25]. Estimation of muscle co-activation has also been investigated to improve the control of prosthetic devices [26–28]. However, the dynamics of muscle co-activation in lower limb amputees during balance controls remains unexplored.

We recorded electromyography (EMG) signals from muscles of the trunk and the lower intact/dominant limb to assess intermuscular coupling during quiet standing. These muscles are involved in the hip and ankle strategies to maintain balance. We used wavelet coherence analysis to investigate three frequency ranges represented by seven specific frequencies. Sub-cortical low-frequency coherence (two frequencies, 7.56 and 19.86 Hz) was investigated as this frequency range was previously correlated with inputs from the reticulospinal tract, which is involved in unconscious postural control during standing [29]. Cortical mid-frequency coherence (two frequencies, 38.26 and 62.63 Hz) was investigated because of its previous association with dynamical balance tasks [30–32] and the Piper rhythm, which is linked to the cortical drive to muscles [33]. High-frequency coherence (three frequencies, 92.90, 129, and 170.90 Hz) was investigated as it reflects synchronized oscillations of motor unit action potentials. Coherence at this high frequency may indicate a precise synchronization of the motor units between two muscles [34].

We hypothesized that intermuscular coupling at these frequency ranges would differ between the group of amputees and the group of able-bodied controls as quiet standing may demand more cognitive attention to maintain balance in patients with a lower limb amputation. Additionally to the coupling strength, we quantified the dynamics of intermuscular coupling over time using the entropic Half-Life (EnHL) [35] as a measure of non-linear changes in the temporal structure of muscle co-activation. We hypothesized that differences in the control strategies to cope with the loss of mechanical stability and sensory feedback would be reflected in the dynamics of intermuscular coupling.

2. Methods

2.1. Participants

Eleven unilateral transfemoral amputees (age: 54.8 ± 10.9 yr.; height: 171 ± 9.5 cm; weight:
Table 1. Demographics of the participants. N.A.: not available at the time of measurement.

| Group   | ID | Age | Gender | Height in cm | Weight in kg | Dominant leg | Years since amputation | Amputated leg | Stump length in cm |
|---------|----|-----|--------|--------------|--------------|--------------|------------------------|---------------|--------------------|
| Amputee | 1  | 49  | F      | 158          | 67           | L            | 34                     | R             | 25                 |
| Amputee | 2  | 61  | F      | 159          | 74           | R            | 41                     | L             | 23                 |
| Amputee | 3  | 68  | M      | 170          | 69           | R            | 51                     | L             | 24                 |
| Amputee | 4  | 56  | M      | 183          | 72           | R            | 32                     | L             | 21                 |
| Amputee | 5  | 56  | M      | 178          | 92           | R            | 38                     | L             | 22                 |
| Amputee | 6  | 55  | M      | 178          | 82           | R            | 37                     | L             | 29                 |
| Amputee | 7  | 63  | M      | 187          | 67           | R            | 35                     | L             | 24                 |
| Amputee | 8  | 66  | F      | 168          | 62           | R            | 52                     | R             | 28                 |
| Amputee | 9  | 28  | F      | 164          | 63           | R            | 22                     | L             | 28                 |
| Amputee | 10 | 53  | F      | 165          | 59           | R            | 52                     | L             | 13                 |
| Amputee | 11 | 48  | F      | 170          | 83           | R            | 5                      | L             | N.A.               |
| Control | 12 | 47  | M      | 189          | 86           | R            | —                      | —             | —                  |
| Control | 13 | 66  | M      | 179          | 93           | L            | —                      | —             | —                  |
| Control | 14 | 32  | M      | 182          | 73           | L            | —                      | —             | —                  |
| Control | 15 | 56  | M      | 169          | 66           | R            | —                      | —             | —                  |
| Control | 16 | 58  | F      | 166          | 67           | R            | —                      | —             | —                  |
| Control | 17 | 53  | M      | 183          | 92           | L            | —                      | —             | —                  |
| Control | 18 | 53  | F      | 154          | 67           | R            | —                      | —             | —                  |
| Control | 19 | 55  | F      | 166          | 60           | R            | —                      | —             | —                  |
| Control | 20 | 63  | F      | 167          | 80           | R            | —                      | —             | —                  |
| Control | 21 | 40  | F      | 164          | 89           | R            | —                      | —             | —                  |
| Control | 22 | 60  | M      | 182          | 70           | R            | —                      | —             | —                  |

71.8 ± 10.1 kg; 5 female and 6 male) and 11 age-matched able-bodied control participants (age: 53.0 ± 10.0 yr.; height: 173 ± 10.7 cm; weight: 76.6 ± 11.7 kg; 6 female and 5 male) participated in this study (table 1). The inclusion criteria for the amputee participants were that they have a unilateral transfemoral amputation and wear a prosthesis with an active knee joint. Participants with neurological, cardiovascular diseases, or other orthopedic conditions were excluded from the study. All participants had normal or corrected-to-normal vision. Limb dominance was determined as the leg that is mainly used for propulsion (forward acceleration of the body's center of gravity). The experimenter verbally asked the participants: 'With which leg are you going first. For climbing stairs, which leg do you move first or with which leg do you step on the first step'. The dominant leg was determined as the leading leg, i.e. the first leg to step forward.

2.2. Experimental design and data acquisition
Bipolar surface EMG signals were recorded from the M. obliquus externus (OE), M. erector spinae (ES), M. tibialis anterior (TA), and M. medial gastrocnemius (MG) (figure 1(A)) at a sampling frequency of 2 kHz, with a gain of 1000 and filtered at 5–500 Hz (MP35, BIOPAC, CA, USA). EMG signals were measured from the dominant leg in the control group and the intact limb in the amputee group. After cleaning with alcohol to remove loose dead skin particles and oils, the electrodes were placed according to the SENIAM project guidelines [36]. To prevent crosstalk between neighboring muscles, dual electrodes with a small and standardized inter-electrode distance of 20 mm were used (Noraxon USA Inc., Scottsdale, Arizona USA). Reference electrodes were placed on the bony part of the medial and lateral malleolus.

EMG signals were recorded during three trials of quiet upright standing, each of 30 s long, with eyes open and eyes closed. Participants were instructed to quietly stand upright with their feet separated to match shoulder-to-shoulder distance and with their arms loosely hanging on the side close to the body. For the eyes open trials, participants were instructed to gaze at a cross mark positioned at eye level at a distance of 1.5 m. The trial order was randomized, with short breaks (approx. 30 s) between each trial.

2.3. Data analysis
EMG data were processed in MATLAB (Release 2020a, MathWorks, Inc., Natick, Massachusetts, USA). The raw EMG data were passed through a wavelet band-pass filter (cut-off frequency: 1 and 500 Hz) and a fourth-order notch filter at 50 Hz as well as at multiples of 50 Hz to remove the harmonics of the power line frequency present in the signal. Subsequently, electrocardiography (ECG) contaminations were attenuated using a custom MATLAB code as follows. First, the ECG content in the EMG data was estimated by searching for peaks over time in the low-frequency portion of the EMG wavelet power spectrum. Within a pre-defined time-window around these peaks, the low-frequency content of the EMG signal (i.e. linked to ECG contaminations) was subtracted from the original EMG signal.
Figure 1. Signal processing workflow for the coherence analysis and the EnHL calculation. (A) We analyze the strength and dynamics of muscle co-activation modulated by vision after a lower limb amputation. (B) Pre-processing step: EMG signals are band-pass filtered at 1–500 Hz, notch filtered at 50 Hz and harmonics, and ECG contaminations are attenuated. (C) The filter-bank consisting of 7 center frequencies is used to decompose the signals into the EMG intensity (D) by convolving the EMG signal with the wavelets gained from equation (1). (E) Two wavelet transformed signals are subjected to equation (4) to compute the wavelet coherency (shown here is a smoothed coherency with a window of 2000 sample points). (F) The frequency-averaged wavelet coherency is being used for EnHL calculations; shown below is the reshaping process for the gradual randomization of the original time series, the numbers indicate the indices of the time series. The transition curve shows the normalized FuzzyEn as a function of the shuffle-time from an organized to an unorganized state. The half-maximal point is then determined as the EnHL.

2.4. Wavelet transform

Time-frequency analysis of the EMG data was implemented following the wavelet analysis previously published [37]. This analysis yields how the energy of the EMG signals varies over time at different discrete frequencies (figure 1(D)). We applied the wavelet transform to each EMG signal to obtain this representation over time and frequency as follows.

By definition, wavelets are short-time oscillatory time series. We used the complex Cauchy wavelet as mother wavelet, with the different wavelets in the frequency space defined by equation (1)

$$F_{\psi}(f, cf, \text{mode}) = \left(\frac{f}{cf}\right)^{\text{mode}} \cdot e^{i \pi \left(\frac{f}{cf} + \text{mode}\right)}.$$  \hspace{1cm} (1)

where $f$ is the frequency range used as index in the equation, $cf$ is the center frequency (i.e. the frequency of maximal amplitude), and mode is a parameter controlling the bandwidth of the wavelet. A set of non-linearly scaled wavelets were then obtained using equations (2)–(3)

$$cf_j = \frac{1}{0.3} \cdot (1.45 + j)^{1.959}.$$  \hspace{1cm} (2)

$$\text{mode}_j = cf_j \cdot 0.3.$$  \hspace{1cm} (3)

We created seven wavelets by indexing equations (2) and (3) with $j = 1, 2, 3, 4, 5, 6$ and 7, which corresponds to $cf = 7.56, 19.86, 38.26, 62.63, 92.90, 129$ and 170.90 Hz (figure 1(C)). Finally, we produced the wavelet transform of the EMG signals by convolving with the filtered EMG signal with each wavelet (figure 1(D)).

2.5. Wavelet coherency

The wavelet coherency between pairs of EMG signals was then computed using the wavelet transform of each EMG signal. In the context of EMG analysis, the coherency measure will be higher if there is a strong co-activation of the two involved muscles, and it will be lower if the co-activation is weaker.

The wavelet spectrum for each center frequency was then subjected to the wavelet coherency [38] defined by equation (4)

$$\text{coherency} = \frac{W_x \cdot W_y}{W_x^{0.5} \cdot W_y^{0.5}}.$$  \hspace{1cm} (4)

In equation (4), $W_x$ and $W_y$ are the wavelet transforms of the two signals $x$ and $y$, with the bar on top indicating their complex conjugate. The wavelet coherency is defined as the product of the wavelet transformed signal $x$ with the conjugate of the wavelet transformed signal $y$, normalized by the product
of the modulus of the wavelet transform of each signal. The complex wavelet coherency was then averaged over time for each center frequency to obtain one coherency value per center frequency. Finally, we used the modulus of the averaged coherency as a measure of the strength of intermuscular coupling.

The coherency values were considered significant if they exceeded a baseline value computed as follows. One of the signals was shifted by one sample point in time and the modulus of the wavelet coherency was computed. This procedure was repeated 1000 times, corresponding to 500 ms. The baseline was defined as the coherency values averaged across all 1000 shifted time series, trials and, participants. In this way, any true coherence caused by common neural input to the two muscles is destroyed because the recorded signals are temporally displaced to each other. What remains can be considered as the coherency of the noise inherent to both signals. This averaged baseline plus two standard deviations resembles the α level of confidence at 95% of the wavelet coherency analysis.

2.6. Dynamics of the wavelet coherency

To investigate changes in muscle co-activation over time, it was first necessary to smooth the wavelet coherency signals in time and frequency. Otherwise, the modulus of the coherency between two signals equals one [39]. Smoothing over time was achieved by applying a moving average filter with a window of 1000 ms (2000 sample points). Smoothing over frequency was implemented by averaging across center frequencies, resulting in three frequency bands, a low-frequency band (average of two frequencies, 7.56–19.86 Hz), a mid-frequency band (average of two frequencies, 38.26–62.63 Hz), and a high-frequency band (average of three frequencies, 92.90, 129 and 170.90 Hz). To analyze how intermuscular coupling changes over time, the modulus was then calculated of the complex coherency (figure 1(D)).

The EnHL was used to investigate the dynamics of the changes in intermuscular coupling over time. When the EnHL is applied to time-frequency EMG-EMG coherency signals, it measures the time-scale at which current coherency values are related to previous ones. A large EnHL value corresponds to a long time-scale, and the longer the time-scale, the slower are the dynamics of the coupling between muscles.

The EnHL was computed as published elsewhere [35] and it is briefly explained as follows. First, the modulus of the coherency is gradually shuffled using the reshape scale method [35] in a way that at each reshaping step the signal became gradually randomized. The Fuzzy Entropy (with parameters $m = 3, r = 0.7, \text{expo} = 5$) of each rescaled time series was then calculated, resulting in a measure of the regularity of each rescaled signal [40]. Thus, with each reshaping of the time series, the entropy increases as the reshaped signals become more random, i.e. the time distance between previously adjacent points increases (figure 1(F)). In total, the Fuzzy Entropy was computed for 30 reshaped signals for each frequency band. The entropy values of each rescale were then normalized with regard to the Fuzzy Entropy value calculated from the total randomized original time series. Finally, the EnHL is defined as the rescale value where the normalized entropy of the time series reaches half its maximum, representing the transition from a regular to a random signal (figure 1(F)).

2.7. Surrogate analysis

Electrophysiological recordings such as EMG signals may exhibit a pattern that appears random yet they have a deterministic origin. To investigate if the EMG-EMG coherency time series differed from signals with a random origin, we created surrogates of the original EMG-EMG coherency time series using the amplitude-adjusted Fourier transform (AAFT) algorithm [41]. The AAFT randomizes the phase of the signal, destroying the information content of the signal while retaining its amplitude characteristics [42, 43]. This allowed us to compare the EnHL of the original time series and the EnHL of its surrogate counterpart to rule out whether the EMG-EMG coherency time series were of non-random origin and contained information originated by physiological processes.

2.8. Statistical analysis

The distributions of the coherence data were estimated to fit a beta distribution and the EnHL values to a gamma distribution [44]. Therefore, the coherence and the EnHL data were fitted to a generalized linear mixed model (GLMM) with the above mentioned distributions.

For the wavelet coherency analysis, we fitted a GLMM for each muscle pair and center frequency. For each model, fixed effects were defined as Group (two levels: amputee, controls), and Condition (two levels: eyes open, eyes closed). The factor Participants was used as a random effect.

For the EnHL values, we fitted a GLMM for each muscle pair and frequency band. For each model, fixed effects were Group and Condition, and we also included a fixed effect of Signal Type (two levels: original and surrogates). After confirming the non-random origin of the time series, we removed the Signal Type effect for subsequent analyses. The factor Participants was also used as a random effect.

Outliers present in the data were removed following the criteria that a data point that lies above the 75th or below the 25th percentile by a factor of 1.5 times the interquartile range is an outlier. Subsequently, an analysis of deviance based on the mixed beta regression was used to investigate any main or interaction effects (i.e. type III Wald Chi-square test).

In the occurrence of a significant main interaction, a follow-up post hoc test using pairwise
comparisons with Holm’s sequential Bonferroni procedure was implemented. The significance level was set to $\alpha = 0.05$. All the statistical analysis was performed using R [45].

3. Results

3.1. Wavelet coherence
The statistical analysis was only performed on the muscle pairs of OE-ES and TA-MG as the other muscle pairs were below or around the baseline representing the significance threshold at which the coherence in our data is considered to be significant (figure 2). An analysis of deviance (type III Wald $\chi^2$-test) based on the GLMM was conducted as a two-factor analysis including each muscle pair and each center frequency. For the muscle pair OE-ES, there was a significant Group effect ($\chi^2 = 4.047, p = 0.044$, table 2) at the center frequency of 19.86 Hz. At the center frequency of 129 Hz for this muscle pair, a two-way interaction was observed between Group x Condition ($\chi^2 = 5.182, p = 0.023$, table 2), which was further analyzed using post-hoc tests with Holm-Bonferroni correction. For the eyes open condition, amputees had significantly higher coupling than controls ($t = 2.444, p = 0.032$, figure 3). The other center frequencies for the muscle pair of OE-ES did not reveal significant main or interaction effects.

The lower leg muscle pair TA-MG did reveal an interaction at 7.56 Hz between Group x Condition ($\chi^2 = 11.175, p < 0.001$, table 2). Post-hoc testing revealed that amputees had significantly higher coupling with visual feedback than without vision ($t = 2.200, p = 0.030$, figure 3), and this effect was reversed in controls ($t = -2.546, p = 0.024$, figure 3). Moreover, for the eyes closed condition it was observed that amputees had significantly lower coupling than controls ($t = -2.305, p = 0.046$, figure 3). At the three middle to high frequencies, a significant Condition effect was observed (62.63 Hz: $\chi^2 = 4.593, p = 0.032$; 92.90 Hz: $\chi^2 = 12.342, p < 0.001$; 129.00 Hz: $\chi^2 = 26.676, p < 0.001$, table 2), showing significantly stronger coupling for the eyes open condition than for the eyes closed condition across groups. For the frequency 170.90 Hz and the muscle pair TA-MG, a significant Condition effect ($\chi^2 = 17.634, p < 0.001$, table 2) and Group x Condition ($\chi^2 = 8.500, p = 0.004$, table 2) were observed. Post-hoc test revealed significantly stronger muscle coupling for amputees than for controls when they had their eyes open ($t = 5.088, p < 0.001$, figure 3). The other center frequencies did not exhibit significant main effects.

3.2. Dynamics of the wavelet coherence
Wald $\chi^2$-test showed that there was a significant Signal Type effect showing that the surrogate data is consistently lower than the original data ($\chi^2 = 251.381, p < 0.001$, table 3). This confirms the non-random origin of the data.

In a succeeding step, the original data were analyzed without the surrogate data and again split up into each frequency band and muscle pair. For the OE-ES, the two-factor analysis revealed a significant main effect for the Group ($\chi^2 = 5.093, p = 0.024$, table 4), indicating that amputees had significantly lower dynamics than controls across conditions (figure 4). The same effect was observed for the muscle pair TA-MG ($\chi^2 = 4.821, p = 0.028$, table 4, figure 4). From the middle frequency band, there was a significant Condition effect only for the TA-MG muscle pair ($\chi^2 = 4.439, p = 0.035$, table 4), showing that for the eyes open condition amputees and controls exhibited higher dynamics than with eyes closed.

4. Discussion
This study aimed to investigate whether unilateral transfemoral amputees exhibit altered muscle co-activation patterns to maintain balance during upright standing. The co-activation of four muscles during quiet standing was measured and quantified via EMG-EMG wavelet coherence analysis. The EnHL of the wavelet coherence in the time domain was computed to investigate the dynamics of inter-muscular coupling. Together, these two measures provided insight into the proportional strength of the muscle co-activations and its dynamics during balance control.

Seven center frequencies were investigated and were grouped into three frequency bands, a low- (7.56 and 19.86 Hz), mid- (38.26 and 62.63 Hz), and high-frequency (92.90, 129, and 170.90 Hz) band. The low-frequency band is associated with sub-cortical/spinal inputs and represents neural pathways correlated with unconscious balance control [29]. Differences between groups and visual conditions at 7.56 Hz were observed for the muscle pair TA-MG, where amputees had significantly stronger coupling during the eyes open condition than during the eyes closed condition. This effect was reversed in controls. Without vision, amputees exhibited significantly lower coupling than controls, suggesting a more unconscious balance control. Further, amputees showed stronger coupling regardless of visual condition at 19.86 Hz for the OE-ES muscle pair. The mid-frequency band reflects cortical inputs related to dynamical motor tasks [30–32]. We considered this frequency band since balance is considered a dynamical task [2]. The higher-frequency band is associated with oscillations in the synchronizations of motor-unit action potentials for fast conducting fibers [34], where we observed a significantly stronger coupling in the TA-MG muscle pair in the group of amputees compared to controls when vision was allowed.
The muscle pairs OE-TA, OE-MG, ES-TA, and ES-MG did not exhibit significant coherency, and thus they seem to not receive strong common neural input during upright standing. This was expected as for our rather simple experimental task, distant muscles most likely do not receive meaningful, strong common neural input. This may be different for more difficult balance tasks, such as a dual task or one including perturbations, where participants must move with a stronger use of force against the
Table 2. Results of the two-way analysis of deviance (type 3 Wald $\chi^2$ tests) applied on the generalized linear mixed beta model of the coherency data. Statistical significances are marked in boldface ($\alpha = 0.05$).

| OE-ES: 7.56 Hz | TA-MG: 7.56 Hz |
|----------------|----------------|
| **Main or interaction** | **Main or interaction** |
| $\chi^2$ | $p$-value | $\chi^2$ | $p$-value |
| Group | 0.346 | 0.557 | Group | 0.127 | 0.721 |
| Condition | 0.037 | 0.848 | Condition | 0.059 | 0.809 |
| Group $\times$ Condition | 3.408 | 0.065 | **Group $\times$ Condition** | 11.175 | <0.001 |

| OE-ES: 19.86 Hz | TA-MG: 19.86 Hz |
|----------------|----------------|
| **Main or interaction** | **Main or interaction** |
| $\chi^2$ | $p$-value | $\chi^2$ | $p$-value |
| Group | 4.047 | 0.044 | Group | 0.186 | 0.666 |
| Condition | 2.213 | 0.137 | Condition | 3.463 | 0.063 |
| Group $\times$ Condition | 0.648 | 0.421 | Group $\times$ Condition | 0.538 | 0.463 |

| OE-ES: 38.26 Hz | TA-MG: 38.26 Hz |
|----------------|----------------|
| **Main or interaction** | **Main or interaction** |
| $\chi^2$ | $p$-value | $\chi^2$ | $p$-value |
| Group | 0.004 | 0.949 | Group | 0.285 | 0.594 |
| Condition | 1.016 | 0.313 | Condition | 0.040 | 0.841 |
| Group $\times$ Condition | 0.978 | 0.323 | Group $\times$ Condition | 0.385 | 0.535 |

| OE-ES: 62.63 Hz | TA-MG: 62.63 Hz |
|----------------|----------------|
| **Main or interaction** | **Main or interaction** |
| $\chi^2$ | $p$-value | $\chi^2$ | $p$-value |
| Group | 0.034 | 0.853 | Group | 0.613 | 0.433 |
| Condition | 2.010 | 0.156 | Condition | 4.593 | 0.032 |
| Group $\times$ Condition | 0.071 | 0.790 | Group $\times$ Condition | 0.339 | 0.561 |

| OE-ES: 92.90 Hz | TA-MG: 92.90 Hz |
|----------------|----------------|
| **Main or interaction** | **Main or interaction** |
| $\chi^2$ | $p$-value | $\chi^2$ | $p$-value |
| Group | 0.611 | 0.434 | Group | 0.065 | 0.799 |
| Condition | 0.620 | 0.431 | **Condition** | 12.342 | <0.001 |
| Group $\times$ Condition | 0.535 | 0.464 | Group $\times$ Condition | 0.798 | 0.372 |

| OE-ES: 129.00 Hz | TA-MG: 129.00 Hz |
|----------------|----------------|
| **Main or interaction** | **Main or interaction** |
| $\chi^2$ | $p$-value | $\chi^2$ | $p$-value |
| Group | 2.787 | 0.095 | Group | 0.166 | 0.683 |
| Condition | 0.044 | 0.834 | **Condition** | 26.676 | <0.001 |
| Group $\times$ Condition | 5.182 | 0.023 | Group $\times$ Condition | 3.321 | 0.068 |

| OE-ES: 170.90 Hz | TA-MG: 170.90 Hz |
|----------------|----------------|
| **Main or interaction** | **Main or interaction** |
| $\chi^2$ | $p$-value | $\chi^2$ | $p$-value |
| Group | 2.097 | 0.148 | Group | 0.491 | 0.484 |
| Condition | 1.912 | 0.167 | **Condition** | 17.634 | <0.001 |
| Group $\times$ Condition | 3.398 | 0.065 | Group $\times$ Condition | 8.500 | 0.004 |

Sway motion of the body. For example, anterior and posterior muscle pairs have been proposed to form anterior and posterior muscle synergies that move the CoP forward and backward, respectively [12]. We, therefore, focused the analysis on the muscle pairs OE-ES and TA-MG, which presented significant coupling, showing the formation of muscle synergies. At the lower frequency band, amputees displayed different dynamics of muscle co-activation than controls. The dynamics of intermuscular coupling did not change between amputees and controls for the middle or high frequencies.

4.1. Amputees and able-bodied controls exhibit different intermuscular coupling at the sub-cortical level modulated by visual feedback

Significant intermuscular coupling was observed at 7.56 Hz in the group of amputees, with significantly stronger coupling for the muscle pair TA-MG when they had their eyes open compared to when
they were closed. This pattern was reversed in the group of controls, i.e. they had a stronger coupling when they closed their eyes. This shows clear differences in the neuromuscular strategies implemented at the sub-cortical level by amputees compared to controls. Intermuscular coupling at this frequency was previously linked with inputs from the reticulospinal tract, which is associated with unconscious balance control [29]. Amputees possibly present a weaker conscious input to muscles when vision is allowed, while they may need to be more consciously focused on maintaining balance when they close their eyes. Moreover, without visual feedback amputees show significantly weaker coupling over this muscle pair than controls, suggesting that amputees more rely on visual information to control their balance through this muscle pair.

4.2. Amputees exhibit stronger intermuscular coupling at the cortical level independent of visual feedback to control the hip strategy

Amputees exhibit stronger co-activation of muscles at 19.86 Hz than controls for the muscle pair OE-ES, implying a stronger focus on the hip strategy.

Table 3. Results of the five-way analysis of deviance (type 3 Wald χ² tests) applied on the generalized linear mixed gamma model of the EnHL data. Statistical significances are marked in boldface (α = 0.05).

| Main or interaction effect            | X²     | p-value |
|--------------------------------------|--------|---------|
| Group                                | 0.042  | 0.837   |
| Muscle Pair                          | 351.487| <0.001  |
| Condition                            | 2.495  | 0.114   |
| Frequency Band                       | 142.756| <0.001  |
| Signal Type                          | 251.381| <0.001  |
| Group × Muscle Pair                  | 5.110  | 0.024   |
| Group × Condition                    | 8.155  | 0.004   |
| Muscle Pair × Condition              | 2.218  | 0.136   |
| Group × Frequency Band               | 13.630 | <0.001  |
| Muscle Pair × Frequency Band         | 376.762| <0.001  |
| Condition × Frequency Band           | 10.026 | 0.007   |
| Group × Muscle Pair × Condition      | 0.540  | 0.462   |
| Group × Muscle Pair × Frequency Band| 1.975  | 0.372   |
| Group × Condition × Frequency Band   | 2.308  | 0.315   |
| Muscle Pair × Condition × Frequency Band | 4.840 | 0.089 |
| Group × Muscle Pair × Condition × Frequency Band | 0.204 | 0.903 |
Table 4. Results of the two-way analysis of deviance (type 3 Wald $\chi^2$ tests) applied on the generalized linear mixed beta model of the EnHL data. Statistical significances are marked in boldface ($\alpha = 0.05$).

| Main or interaction | OE-ES: low frequency band | TA-MG: low frequency band |
|---------------------|---------------------------|----------------------------|
|                     | $\chi^2$ | $p$-value | $\chi^2$ | $p$-value |
| Group               | 5.093   | 0.024     | 4.821   | 0.028   |
| Condition           | 0.220   | 0.639     | 3.829   | 0.050   |
| Group $\times$ Condition | 1.280   | 0.258     | 3.496   | 0.062   |

| Main or interaction | OE-ES: middle frequency band | TA-MG: middle frequency band |
|---------------------|-------------------------------|-------------------------------|
|                     | $\chi^2$ | $p$-value | $\chi^2$ | $p$-value |
| Group               | 0.544   | 0.461     | 0.548   | 0.459   |
| Condition           | 0.809   | 0.368     | 4.439   | 0.035   |
| Group $\times$ Condition | 0.760   | 0.383     | 1.123   | 0.289   |

| Main or interaction | OE-ES: high frequency band | TA-MG: high frequency band |
|---------------------|-----------------------------|-----------------------------|
|                     | $\chi^2$ | $p$-value | $\chi^2$ | $p$-value |
| Group               | 0.301   | 0.583     | 0.285   | 0.593   |
| Condition           | 0.360   | 0.548     | 0.759   | 0.384   |
| Group $\times$ Condition | 0.082   | 0.775     | 0.153   | 0.696   |

Figure 4. Interaction of the EnHL of the wavelet coherency over time. Significant differences between groups are displayed as # and differences between conditions are marked with +. For each group there were 11 participants and each trial was repeated three times (#+/ $p < 0.05$). Error bars are SEM.

Amputees displayed a larger medio-lateral sway, dominated by this balance strategy [16]. This frequency band has been associated using corticomuscular coherence with isometric contractions and more cortical input [46]. This indicates also a more stringent control over the muscles, which seems to be independent of visual condition.

4.3 Amputees but not able-bodied controls exhibit different intermuscular coupling at higher frequencies modulated by visual feedback

For the muscle pair OE-ES at 129 Hz, amputees displayed stronger coupling than controls with visual feedback. For the muscle pair TA-MG at 170.90 Hz, amputees exhibited a significantly stronger coupling.
while visual feedback was allowed compared to the control group. At these frequencies, EMG-EMG coherency results from fast oscillations that synchronize streaming from motor units with high conduction velocities [34], and it is also associated with the shape of the action potential of the motor units [47, 48]. It has been suggested that during movement, the CNS select muscle fibers with fast and slow conducting properties [49]. Thus, fast-twitching muscle fibers may receive higher common neural input in amputees. Fast twitching fibers generate MUAPs of shorter duration. It was observed that a higher content of fast-twitch fibers may be associated with the ability to produce rapid forces and to adjust to perturbations [50]. While this requires further investigations, it might be the case that amputees had to readjust their sway more often than controls to maintain a quiet standing, involving rather fast conducting muscle fibers indicating overall the mal adapted approach to maintain balance in lower-limb amputees. At the frequency of 62.63, 92.90, and 129.00 Hz for the muscle pair TA-MG and regardless of the group, the coherency values were in general higher with visual feedback than without vision. These frequencies are not only associated with cortical drives to muscles, involved in rather strong, voluntary, and slow contractions [51], but also reflects the above mentioned synchronization and shape of action potentials [34, 47, 48].

4.4. Amputees exhibit lower dynamics of muscle co-activation at the sub-cortical level modulated by visual feedback

Amputees presented significantly lower dynamics of muscle co-activation than able-bodied controls for both muscle pairs independent of visual condition at the low-frequency band (7.56–19.86 Hz, figure 4). Thus, amputees exhibited a different co-activation time scale than able-bodied controls, possibly because of the lack of sensory feedback from the amputated leg, which is required for a more frequent updating of the neuromuscular control over the muscles. Activity at this low frequency has been previously correlated with inputs from the reticulospinal tract, which is involved in unconscious postural control during standing [29]. Proprioceptive feedback is the most sensitive and the fastest sensory input to perceive changes in postural sway [52]. While the effect of vision was not statistically significant ($p > 0.05$), with a larger sample size it may be confirmed that this difference in the dynamics of muscle co-activation is mainly during the eyes open condition (figure 4). Compared to able-bodied controls, the intervention of the postural control to co-activate muscles in amputees became less frequent when vision was allowed, suggesting a less frequent activation during unconscious balance control. Removing vision would eliminate the difference in the dynamics of the muscle co-activation between amputees and controls, suggesting again that the incongruency between the lack of somatosensory and visual information plays an important role in the control of posture of lower limb amputees.

4.5. Limitations

Our experimental protocol may have not been challenging enough as the task of upright quiet standing did not involve external perturbations. For instance, it has been shown that with increasing balance task difficulty, intermuscular coupling at the mid-frequency band increases for agonist-agonist muscle pairs [53]. Future work should focus on perturbed standing to further explore the role of sensory feedback on balance control after amputation. Adding a more cognitive demanding task such as a dual task could also provide more information regarding intermuscular coupling at the cortical level [54]. For such tasks, measurements of cognitive load and error signals may be taken into account. Further, the population in this study was skewed toward older individuals (>50 years old) and neuromuscular adaptations may differ in younger participants. Even though we controlled this factor by including age-matched controls, further investigations on the dynamics of intermuscular coupling should be performed in a younger population of amputees and able-bodied individuals. While the stump length of the amputee patients recruited for this study did not differ for a large margin (table 1), this together with differences in the structure of the residual muscles may affect balance control [55]. Hence, they represent another potential limiting factor of our study. Finally, since the probability of unexpected falls during perturbations and under cognitive load increases with ageing, results could also help to better understand these accidents and develop prevention strategies for our ageing societies.

4.6. Conclusion

The results of our study indicate the development of neuromuscular adaptations reflected in the proportional strength and in dynamics of intermuscular coupling at different organizational levels of the nervous system. These adaptations were overall modulated by visual feedback and point to neurophysiological changes occurring after a lower limb amputation. This study evidences the need of further understanding the neuromechanical adaptations happening after an amputation to improve the control strategies and neural stimulation paradigms in the next generations of bionic limbs. We believe that this approach could be complementary to transfer learning approaches, which showed improved calibration times for myoelectric controlled prosthetic devices [56, 57].
Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Acknowledgments

This work was supported by the German Federal Ministry of Education and Research (INOPRO project, 16SV7656) and the Wissenschaftliche Gesellschaft Freiburg. This work is part of BrainLinks-BrainTools which was funded by the German Research Foundation (DFG, grant no. EXC 1086) and is currently funded by the Federal Ministry of Economics, Science and Arts of Baden-Württemberg within the sustainability program for projects of the excellence initiative II. We express our sincerest thanks to all amputee subjects and able-bodied participants of this study. We also thank Dr. Nina Stobbe for her help with preparing the ethical approval documents of this study. We thank Prof. Dr. Peter Deibert for allowing us to perform the experiments in his laboratory. We also thank Elana Goldenkoff for initiating this project during her short visit. We thank Dr. Danesh Ashouri for designing part of the graphical content.

Ethical statement

Each participant signed a written informed consent form before performing the experiment, which was approved by the local ethics committee of the University of Freiburg (ethical approval N°230/18).

ORCID iDs

C Mehring C Mehring https://orcid.org/0000-0001-8125-5205
C Pasluosta https://orcid.org/0000-0001-5335-9840

References

[1] Horak F B 2006 Postural orientation and equilibrium: what do we need to know about neural control of balance to prevent falls? Age Ageing 35 7–11
[2] Balasubramaniam R and Wing A M 2002 The dynamics of standing balance Trends Cogn. Sci. 6 531–6
[3] Winter D 1995 Human balance and posture control during standing and walking Gait Posture 3 193–214
[4] Park K H, Lim J Y and Kim T H 2016 The effects of ankle strategy exercises on unstable surfaces on dynamic balance and changes in the COP J. Phys. Ther. Sci. 28 456–9
[5] Ting L H 2007 Dimensional reduction in sensorimotor systems: a framework for understanding muscle coordination of posture Prog. Brain Res. 165 299–321
[6] Winter D A, Prince F, Frank J S, Powell C and Zabjek K F 1996 Unified theory regarding A/P and M/L balance in quiet stance J. Neurophysiol. 75 2334–43
[7] Horak F B and Nashner L M 1986 Central programming of postural movements J. Neurophysiol. 55 1369–81
[8] D’Avella A and Bizzi E 2005 Shared and specific muscle synergies in natural motor behaviors Proc. Natl Acad. Sci. USA 102 3676–81
[9] Ting L H and Macpherson J M 2005 A limited set of muscle synergies for force control during a postural task J. Neurophysiol. 93 609–13
[10] Torres-Oviedo G and Ting L H 2007 Muscle synergies characterizing human postural responses J. Neurophysiol. 98 2144–56
[11] Bernshtein N A 1967 Co-ordination and Regulation of Movements (Oxford: Pergamon) (https://doi.org/B0007TIDZK)
[12] Krishnamoorthy V, Latash M L, Scholz J P and Zatsiorsky V M 2003 Muscle synergies during shifts of the center of pressure by standing persons Exp. Brain Res. 152 281–92
[13] Krishnamoorthy V, Zatsiorsky V M, Latash M L and Scholz J P 2004 Muscle modes during shifts of the center of pressure by standing persons: effect of instability and additional support Exp. Brain Res. 157 18–31
[14] Danna-Dos-Santos A, Shapkova E Y, Shapkova A L, Degani A M and Latash M L 2009 Postural control during upper body locomotor-like movements: similar synergies based on dissimilar muscle modes Exp. Brain Res. 193 565–79
[15] Beltran E J, Dingwell J B and Wilken J M 2014 Margins of stability in young adults with traumatic transtibial amputation walking in destabilizing environments J. Biomech. 47 1138–43
[16] Petrofsky J S and Khowailed I A 2014 Postural sway and motor control in trans-tibial amputees as assessed by electroencephalography during eight balance training tasks Med. Sci. Monit. 20 2695–704
[17] Arifin N, Azuan N, Osman A, Ali S, Gholizadeh H, Abu W and Wan B 2014 Postural stability characteristics of transtibial amputees wearing different prosthetic foot types Sci. World J. 2014 1–6
[18] Geurts A C H and Mulder T W 1992 Reorganisation of postural control following lower limb amputation: theoretical considerations and implications for rehabilitation Physiother. Theory Pract. 8 145–57
[19] Barnett C T, Vanicek N and Polman R C J 2013 Postural responses during volitional and perturbed dynamic balance tasks in new lower limb amputees: a longitudinal study Gait Posture 37 230–35
[20] Fuchs K, Krauskopf T, Lauck T B, Klein L, Mueller M, Herget G W, Von Tschcharner V, Stutzig N, Stiegzit T and Pasluosta C 2021 Influence of augmented visual feedback on balance control in unilateral transfemoral amputees Front. Neurosci. 15 1–11
[21] Valle G, Salji G, Fogle E, Cimolato A, Pettrini F M and Raspopovic S 2021 Mechanisms of neuro-robotic prosthesis operation in leg amputees Sci. Adv. 7 eabd8354
[22] Pettrini F M et al 2019 Sensory feedback restoration in leg amputees improves walking speed, metabolic cost and phantom pain Nat. Med. 25 1356–63
[23] Charkhkar H, Christie B P and Triolo R J 2020 Sensory neuroprosthesis improves postural stability during sensory organization test in lower-limb amputees Sci. Rep. 10 69894
[24] Claret C R, Herget G W, Koura L, Wiest D, Adler J and Von Tschcharner V 2019 Neuromuscular adaptations and sensorimotor integration following a unilateral transfemoral amputation J. NeuroEng. Rehabilitation 11 1–11
[25] Xu Z, Yan F, Chen T L, Zhang M, Wong D W C, Jiang W T and Fan Y B 2021 Non-amputated limb muscle coordination of unilateral transfemoral amputees J. Biomech. 115 110155
[26] Fleming A, Stafford N, Huang S, Hu X, Ferris D P and Huang H H 2021 Myoelectric control of robotic lower limb prosthesis: a review of electromyography interfaces, control paradigms, challenges and future directions J. Neural Eng. 18 041003
[27] Hargrove L J, Simon A M, Young A J, Lipschutz R D, Finucane S B, Smith D G and Kuiken T A 2013 Robotic leg control with EMG decoding in an amputee with nerve transfers New Engl. J. Med. 369 1237–42
