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The Existence of Shared Muscle Synergies Underlying Perturbed and Unperturbed Gait Depends on Walking Speed

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Abstract: Muscle synergy theory assumes that the central nervous system generates a wide range of complex motor outputs by recruiting muscle synergies with different strengths and timings. The current understanding is that a common set of muscle synergies underlies unperturbed as well as perturbed walking at self-selected speeds. However, it is not known whether this is the case for substantially slower walking. The aim of this study was to investigate whether a shared set of muscle synergies underlies balance recovery responses following inward- and outward-directed perturbations in the mediolateral direction at various perturbation onsets and walking speeds. Twelve healthy subjects walked at three walking speeds (0.4, 0.6, and 0.8 m/s) on a treadmill while perturbations were applied to the pelvis using the balance assessment robot. A set of sixteen EMG signals, i.e., eight muscles per leg, was measured and decomposed into muscle synergies and weighting curves using non-negative matrix factorization. The muscles included were left and right tibialis anterior, soleus, gastrocnemius medialis, gastrocnemius lateralis, rectus femoris, hamstring, gluteus medius, and gluteus maximus. In general, four muscle synergies were needed to adequately reconstruct the data. Muscle synergies were similar for unperturbed and perturbed walking at a high walking speed (0.8 m/s). However, the number of similar muscle synergies between perturbed and unperturbed walking was significantly lower for low walking speeds (0.4 and 0.6 m/s). These results indicate that shared muscle synergies underlying perturbed and unperturbed walking are less present during slow walking compared to fast walking.

Keywords: perturbed walking; electromyography; non-negative matrix factorization; muscle synergies; gait speed; balance control

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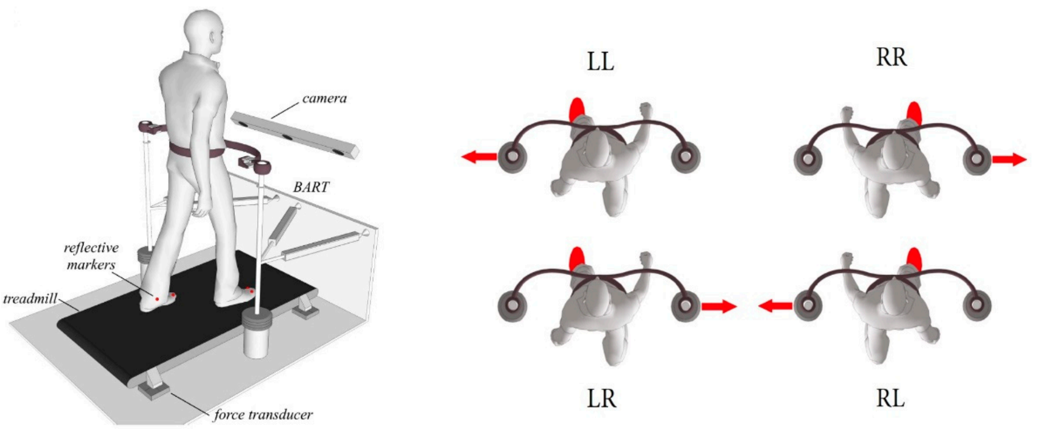
1. Introduction

Several studies support the conception that a small set of muscle synergies underlies walking [1–3]. Electromyography (EMG) data of a set of muscles can be decomposed into a smaller set of synergies by a dimensionality reduction algorithm such as the frequently used non-negative matrix factorization (NNMF). Typically, four muscle synergies have been shown to sufficiently explain the activity of lower limb muscles during gait at a self-selected speed in healthy subjects. The characteristics of these muscle synergies are described by Clark et al. [2] as follows. The weight acceptance muscle synergy primarily consists of the activity of the gluteus maximus, which extends and abducts the hip, and is mostly active during the initial contact of the stance phase. The propulsion muscle synergy mainly involves the calf muscles soleus and medial gastrocnemius and is mainly active during the late stance. The early swing muscle synergy is mostly active during toe-off, and predominantly consists of the activity of the tibialis anterior and the rectus femoris. The late swing muscle synergy primarily involves hamstrings during terminal swing and initial contact. Chvatal et al. [3] investigated whether these similar synergies were also used when counteracting balance perturbations during walking. They demonstrated that a common set of synergies underlies both perturbed and unperturbed walking for intermediate and high walking speeds.

Various types of perturbations can disturb the cyclical way of walking. Different factors, including perturbation modality, perturbation onset, amplitude, direction, and walking speed can induce different responses to restore balance [4–6]. An important strategy to restore balance is the ankle strategy, in which the center-of-pressure under the stance leg is displaced in the direction of the perturbation [4]. The ankle strategy is regularly followed by the main dynamic balancing response known as the stepping strategy, in which the foot of the leg in swing is placed at an adjusted location to re-establish balance [7]. Another strategy is the hip strategy, which is an inertial strategy in which limb segments are rotated to change the body's angular momentum [4,8].

Matjačić et al. [8] investigated the influence of walking speed on balance strategy selection after perturbations were applied in the mediolateral direction. Results indicated that the stepping strategy was induced for inward-directed perturbations, independent of walking speed (see Figure 1 for the definition of perturbation directions). Contradictorily, walking speed did significantly affect the balance strategy for outward-directed perturbations. At the highest walking speed examined (0.8 m/s), the stepping strategy was mainly used. However, at the lowest walking speed examined (0.4 m/s), the hip strategy, instead of the stepping strategy, dominated the balance response. For the intermediate walking speed (0.6 m/s), the contribution of the hip strategy decreased while the contribution of the stepping strategy increased. Whether these differences in the recruitment of balance strategies at different speeds were also accompanied by changes in the recruitment of muscle synergies was not assessed.



(**a**) (**b**)

Figure 1. The experimental setup and perturbation directions. (**a**) A graphical illustration of the experimental setup. Subjects walked on an instrumented treadmill while receiving pelvic perturbations induced by the BART device. (**b**) A top-view illustration of the perturbation directions. Outward perturbations LL/RR: perturbation to the left/right triggered at left/right foot contact; inward perturbations LR/RL: perturbation to the right/left triggered at left/right foot contact.

The aim of the current study was to investigate the existence of a shared set of muscle synergies underlying balance responses assessed in a group of healthy subjects, following inward- and outward- directed perturbations in the mediolateral direction at various perturbation onsets and walking speeds. A study by Chvatal et al. [3], examining muscle synergies underlying perturbation induced dynamic balance responses during gait, has demonstrated that individuals use a common set of synergies in perturbed and unperturbed walking. However, this study was performed for intermediate and high walking speeds (around 1.2 m/s). To the best of our knowledge, no study has examined muscle synergies underlying unperturbed and perturbed gait at walking speeds between 0.4 and 0.8 m/s.

The biomechanical outcomes in the study by Matjačić et al. [8] indicated that balance strategies during slow walking (0.4 m/s) typically deviate from those during faster walking. Therefore, we hypothesized that a shared set of muscle synergies underlying both perturbed and unperturbed walking would be less present at substantially slower walking speeds compared to faster walking speeds.

2. Materials and Methods

2.1. Experimental Procedure

Twelve healthy male subjects (age: 32.9 ± 6.9 years, height: 79.2 ± 3.7 cm, mass: 78.8 ± 6.6 kg) volunteered in this study. Participants had no neurological or orthopedic impairments. All participants signed informed consent forms. The experiments were performed at the University Rehabilitation Institute Republic of Slovenia. The Slovenian National Ethics Committee approved the study.

The experimental setup consisted of the Balance Assessment Robot for Treadmill walking (BART) (Figure 1) [8,9]. Here, only a brief description of the experimental setup is given, as a more detailed description is provided elsewhere [10,11]. The BART was composed of a pelvic manipulator and an instrumented treadmill. The pelvic manipulator consisted of an actuated pelvic link with pelvic brace. The actuated pelvic link interacted with the subject's pelvis. This haptic interaction was admittance controlled in transparent mode during unperturbed walking (i.e., minimal interaction forces were provided, and the pelvis could be moved freely while walking) and controlled to deliver perturbing forces to the subject's pelvis. These pelvic perturbations could be applied in anteroposterior and mediolateral directions at various moments in the gait cycle. Three walking trials on the treadmill were performed at three different walking speeds (0.4 m/s, 0.6 m/s, and 0.8 m/s). Each separate trial started with a period of unperturbed walking for three minutes, followed by a period of perturbed walking. Right-aligned perturbations were delivered to the pelvis with the right leg entering the stance phase. In case of left-aligned perturbations, the left leg entered the stance phase. Both left- and right-aligned perturbations were either directed inward or outward (Figure 1). The perturbations were applied at three different perturbation onsets (0%, 30%, and 60% of the stance phase of the gait cycle) and with three different perturbation amplitudes (5%, 10%, and 15% of the subject's body weight). Only the data for the perturbation amplitude of 10% of the subject's body weight were included in the current study. Each condition (four directions, three onsets, three amplitudes) was repeated seven times. This resulted in 252 perturbations per trial that were block randomized. The pause between two consecutive perturbations varied randomly from six to eight seconds. A perturbation was delivered to the subject's pelvis at a force impulse of 150 ms. Preceding the actual trials, all subjects were familiarized with walking on the treadmill and receiving perturbations.

2.2. Data Recording and Processing

EMG signals were recorded with a sample frequency of 1 kHz using surface EMG electrodes placed on cleaned skin sites. Muscles included in the EMG data collection were left and right leg tibialis anterior (LTA, RTA), soleus (LSOL, RSOL), gastrocnemius medialis (LGASM, RGASM), gastrocnemius lateralis (LGASL, RGASL), rectus femoris (LRF, RRF), hamstring (LHAM, RHAM), gluteus medius (LGMED, RGMED), and gluteus maximus (LGMAX, RGMAX). For subjects H6 to H12, hip adductors (LADD, RADD) were included instead of GASL. Recordings of the center-of-pressure were obtained by means of four precision force transducers placed underneath the treadmill.

Raw EMG data were Notch filtered (49–61 Hz) and band pass filtered (20–300 Hz) to remove the mean value and noise. Subsequently, the EMG data were full-wave rectified and filtered with a moving average window of 150 ms to obtain EMG envelopes. The data were segmented into gait cycles defined as the period between two consecutive heel strikes, as detected from the center-of-pressure recordings. For left-aligned perturbations, left heel strikes defined the gait cycle. For right-aligned perturbations, right heel strikes defined the gait cycle. Data for each gait cycle were resampled to a 0–100% range consisting of 200 samples.

2.3. Non-Negative Matrix Factorization

For each subject and each condition, a separate data matrix was constructed. Each data matrix contained 16 rows with each row corresponding to one muscle. Three repetitions of the perturbed step cycle were concatenated end to end to construct the data matrix of size m muscles $\times n$ samples. Each row was normalized to the corresponding row's maximum value for unperturbed walking, and each row was normalized to unit variance to weight variations in each muscle equally in the factorization by dividing all entries in the row by this row's standard deviation [12]. The normalization to unit variance was undone after factorization by multiplying with the standard deviation again.

Muscle synergy theory suggests that humans control muscle synergies (i.e., muscle groups) instead of individual muscles to perform movements, assuming that a set of measured muscle activation signals (EMG signals) is a linear combination of underlying patterns [3,12]. A frequently used technique for the decomposition of a set of measured data ($\mathbf{M}$) is NNMF, which factorizes $\mathbf{M}$ into so-called muscle synergies ($\mathbf{W}$) and weighting curves ($\mathbf{C}$):

$$\mathbf{M}_{m\times n} = \mathbf{W}_{m\times s}\mathbf{C}_{s\times n} + \mathbf{E}, \tag{1}$$

where m is the number of muscles, n the number of samples, s the number of synergies, and $\mathbf{E}$ is the error. Muscle synergies represent the relative activation level of the muscles, i.e., the spatial patterns, and weighting curves represent the time-varying weighting coefficients of the muscle synergies, i.e., the temporal patterns. Other common names for muscle synergies and weighting curves are motor modules and motor tuning curves, respectively. Each muscle synergy (column vector $\mathbf{W}_i$) is thus fixed across time and is recruited (i.e., multiplied) by the corresponding weighting curve (row vector $\mathbf{C}_i$). Reconstructed muscle activation patterns decomposed in s synergies, could thus be represented by:

$$\mathbf{M}_{\text{reconstructed}} = \mathbf{W}_1\mathbf{C}_1 + \mathbf{W}_2\mathbf{C}_2 + \ldots + \mathbf{W}_s\mathbf{C}_s. \tag{2}$$

Factorization was performed using the NNMF Matlab function (version 2020a). This function uses a multiplicative update algorithm that starts with random initial values for the non-negative factors $\mathbf{W}$ and $\mathbf{C}$ for every repetition. Factorization was repeated 50 times to avoid local minima. The function minimizes the mean square residual between matrix $\mathbf{M}$ and matrix $\mathbf{W}\cdot\mathbf{C}$. The termination tolerance of the change in size of the residual was 10^{-6}, the termination tolerance of the relative change in the factors $\mathbf{W}$ and $\mathbf{C}$ was 10^{-4}, and the maximum number of iterations was 1000.

2.4. Statistical Analysis

2.4.1. Variance Accounted For

The Variance Accounted For (VAF) was used as a measure of goodness-of-fit to quantify how well the reconstructed EMG data explained the original EMG data. The VAF is defined as 1 − (sum of squared error)/(total sum of squares). The overall VAF ($\text{VAF}_{\text{overall}}$) and the VAF per individual muscle k ($\text{VAF}_{\text{muscle }k}$) are given by:

$$\text{VAF}_{\text{overall}} = 100\% \cdot \left(1 - \frac{\sum_{k=1}^{m}\sum_{j=1}^{n}\left(\mathbf{M}_{k,j} - \mathbf{WC}_{k,j}\right)^2}{\sum_{k=1}^{m}\sum_{j=1}^{n}\left(\mathbf{M}_{k,j}\right)^2}\right) \tag{3}$$

$$\text{VAF}_{\text{muscle }k} = 100\% \cdot \left(1 - \frac{\sum_{j=1}^{n}\left(\mathbf{M}_{k,j} - \mathbf{WC}_{k,j}\right)^2}{\sum_{j=1}^{n}\left(\mathbf{M}_{k,j}\right)^2}\right), \tag{4}$$

where k represents the kth of m muscles and where j represents the jth of n samples of the original measured data set **M** and the reconstructed data set **WC**.

Before performing NNMF, the number of synergies to be extracted needed to be specified based on the VAF [3]. Therefore, one to eight synergies were extracted to decide on the number of synergies needed to sufficiently explain the data based on the criteria for the $\mathrm{VAF}_{\mathrm{overall}}$ and $\mathrm{VAF}_{\mathrm{muscle}\ k}$ [3]:

$$\mathrm{VAF}_{\mathrm{overall}} \geq 85\% \tag{5}$$

$$\mathrm{VAF}_{\mathrm{muscle}\ k} \geq 75\%. \tag{6}$$

The minimum number of synergies that satisfied both requirements for all subjects and conditions was chosen to be the fixed number of synergies for further analyses.

2.4.2. Similarity

Pearson's correlation coefficient (r_p) was used to quantify the similarity between two muscle synergies, each having N scalar values. The definition of r_p is the standardized covariance, i.e.,:

$$r_p(A, B) = \frac{1}{N-1} \sum_{h=1}^{N} \left(\frac{A_i - \mu_A}{\sigma_A} \right) \left(\frac{B_i - \mu_B}{\sigma_B} \right), \tag{7}$$

where A and B are the two muscle synergy vectors to be compared, and where μ and σ are the mean and standard deviation of a muscle synergy vector, respectively [12]. With $n = 16$ muscles, the degrees of freedom was $df = n - 2 = 14$. From the table with critical values, the critical value was $r_{p_{\mathrm{crit}}} = 0.623$ for a level of significance of $\alpha = 0.01$ [3,13]. For $r_p \geq r_{p_{\mathrm{crit}}}$, the observed r_p value was statistically significant and the null hypothesis, i.e., no correlation between the two muscle synergies existed, was rejected [13]. Thus, for $r_p \geq 0.623$, a pair was considered to be similar.

2.4.3. Perturbed versus Unperturbed Walking

To examine whether a common set of muscle synergies underlies perturbed and unperturbed walking, four muscle synergies and weighting curves were extracted for all left-aligned conditions. We only analyzed these conditions, as a preliminary analysis revealed the absence of substantial differences between left- and right-aligned perturbations. The number of perturbed muscle synergies that was similar to the corresponding four unperturbed muscle synergies was determined for each perturbation condition and walking speed separately. Similarity was assessed with Pearson's correlation coefficient r_p. The number of similar muscle synergies between perturbed and unperturbed walking found per condition was averaged across subjects. Eventually, the summative count of each individual synergy that was similar to the corresponding unperturbed synergy was computed over all subjects.

2.4.4. Statistical Tests

One-way repeated measures ANOVAs (factor: perturbation onset, levels: the unperturbed and the three perturbed conditions with different onsets) followed by Bonferroni post hoc tests were performed for each walking speed and perturbation direction separately to determine whether there were significant differences in the number of similar synergies between perturbed and unperturbed walking across subjects. The significance level of the tests was $\alpha = 0.05$.

3. Results

All subjects walked on the treadmill at three different walking speeds, receiving a variety of perturbations while EMG signals were recorded (Figure 2).

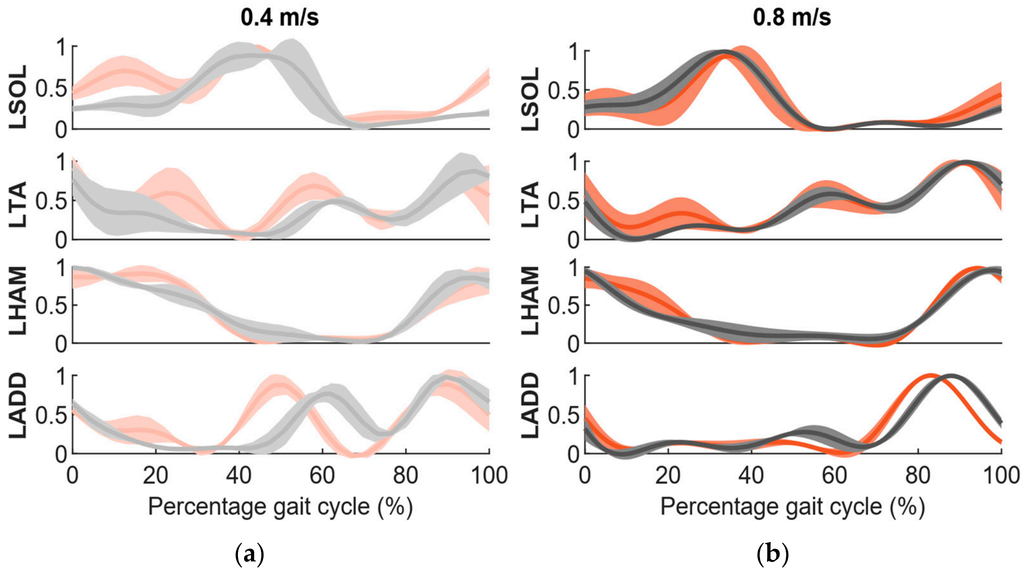


Figure 2. EMG signals for a selection of muscles of a representative subject for inward-directed perturbed (orange) and unperturbed (grey) (**a**) slow and (**b**) fast walking. Perturbation onset was 0% of the stance phase of the gait cycle. EMG signals are shown as the mean (solid line) ± standard deviation (shaded area) of three trials. LSOL, left soleus; LTA, left tibialis anterior; LHAM, left hamstring; LADD, left hip adductor.

Four synergies were sufficient to satisfy the requirement for the overall reconstruction quality ($VAF_{overall} \geq 85\%$) for all left-inward-directed perturbation conditions (Figure 3). This requirement was satisfied for four synergies for all subjects for all conditions, apart from three exceptions in which $VAF_{overall}$ was $83.9 \pm 4\%$. Four synergies were also sufficient to satisfy the requirement for $VAF_{muscle\ k} \geq 75\%$, apart from 1% of the cases where $VAF_{muscle\ k}$ was $69.3 \pm 6\%$.

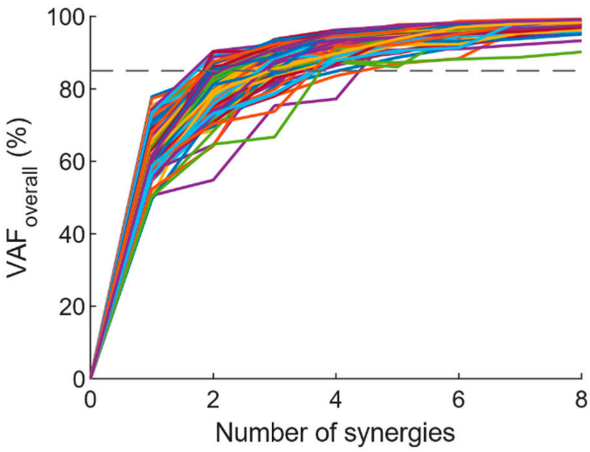
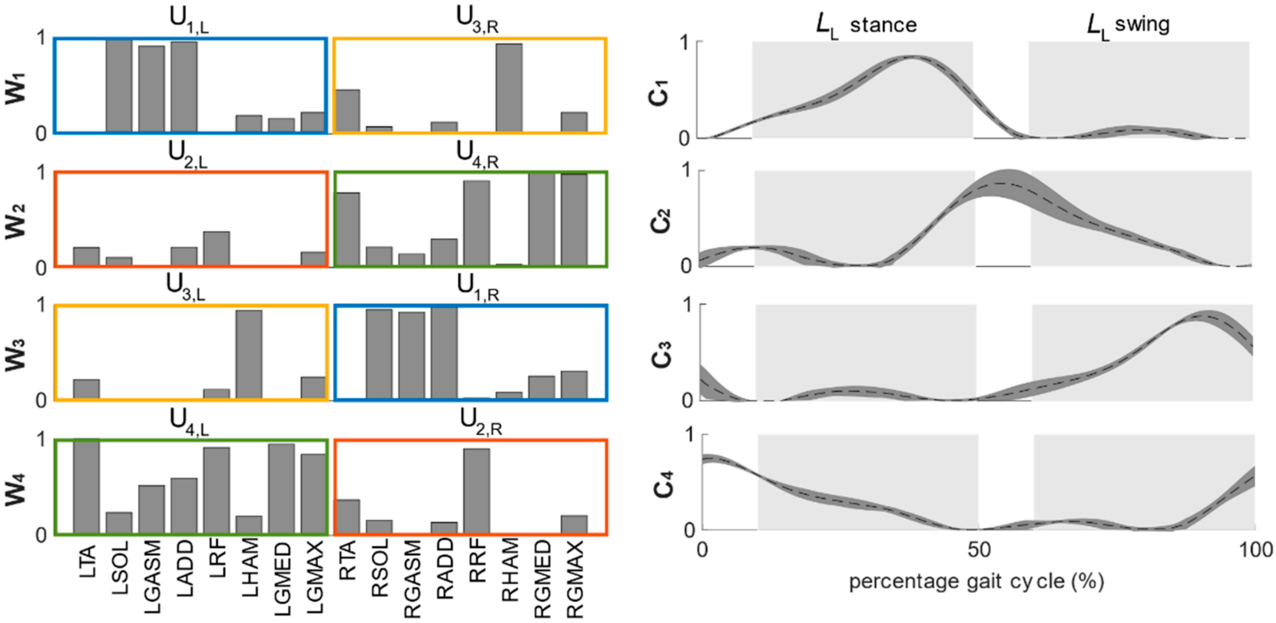


Figure 3. Overall Variation Accounted For ($VAF_{overall}$) with respect to the number of synergies extracted. The graph represents the VAF values for both unperturbed and left-inward perturbed walking for all combinations of walking speed and perturbation onset for all subjects.

All four bilateral muscle synergies were typically composed of one unilateral muscle synergy for each leg (Figure 4). Unilateral muscle synergies were interchanged within the bilateral muscle synergies. Bilateral muscle synergy W_1 was composed of unilateral muscle synergy U_1 of the left leg and unilateral muscle synergy U_3 of the right leg. Contrarily, bilateral muscle synergy W_3 was composed of unilateral muscle synergy U_3 and U_1 of the left and right leg, respectively. Bilateral muscle synergies W_2 and W_4 were composed similarly.

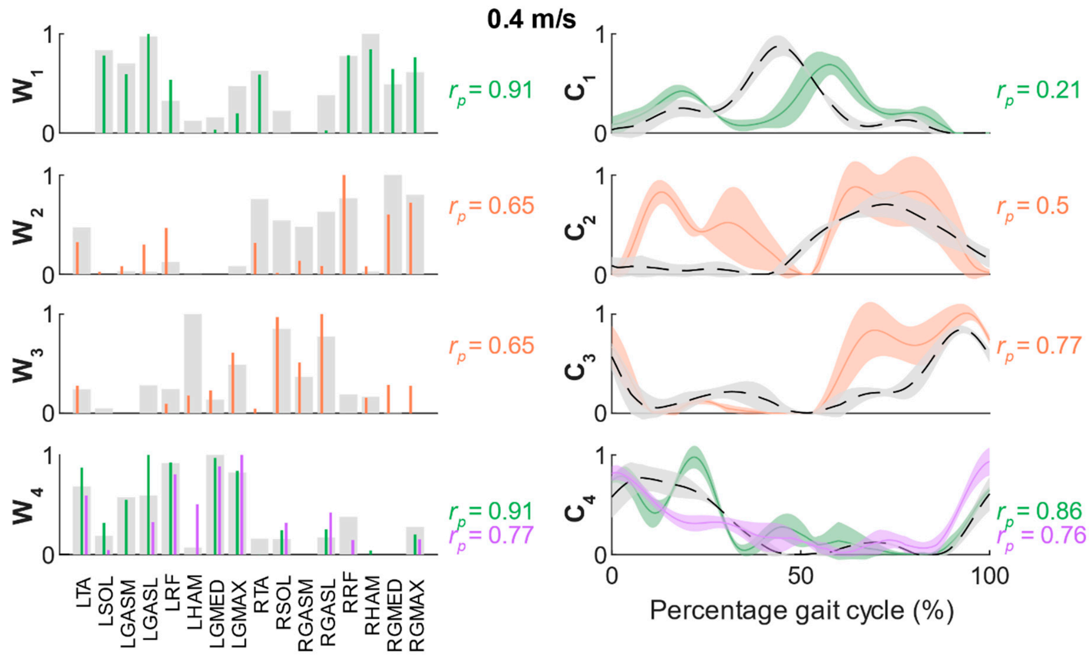


(**a**) (**b**)

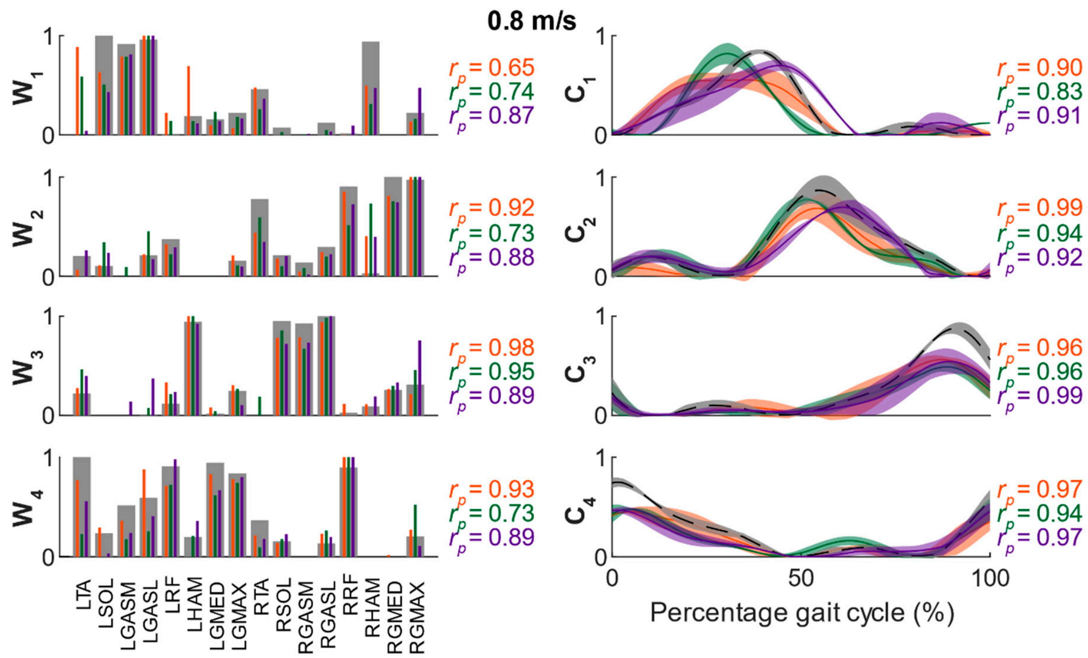
Figure 4. Muscle synergies and weighting curves for unperturbed walking at the highest walking speed for a representative subject. (**a**) Bilateral muscle synergies ($\mathbf{W}_i$) were composed of two unilateral muscle synergies (U_i). (**b**) Weighting curves ($\mathbf{C}_i$) are shown as the mean (solid line) ± standard deviation (shaded area) of three trials. The grey rectangular areas indicate the swing and stance phases of the left leg (L_L).

Unilateral muscle synergy U_1 was named the *push-off* synergy, since its main contributors, the soleus, gastrocnemius medialis, and gastrocnemius lateralis, provide push-off in the terminal stance phase [5,14]. Unilateral muscle synergy $U_2$was named the *initial swing* synergy, since its main contributors, the tibialis anterior and rectus femoris, provide hip flexion to propel the leg forward, and knee extension to stop the knee flexion resulting from the earlier push-off during the initial swing. Unilateral muscle synergy U_3 was named the *terminal swing* synergy, since its major contributors, the tibialis anterior and hamstrings, slow down the swinging leg to prepare the leg for the next stance phase during the terminal swing phase. Unilateral muscle synergy U_4 was named the *loading response* synergy, since it was mostly active during early stance, where its main contributors, the rectus femoris, gluteus medius, and gluteus maximus provide stability.

For a single representative subject, four muscle synergies for inward and outward perturbed fast walking were similar to those for unperturbed fast walking at 0.8 m/s, regardless of perturbation onset $r_p \geq 0.623$ (see Figure 5 for outward-directed perturbations and Figure 6 for inward-directed perturbations). Furthermore, all four weighting curves for perturbed fast walking were similar to those for unperturbed fast walking as well. Contradictorily, only one or two muscle synergies per condition for perturbed slow walking were similar to those for unperturbed slow walking at 0.4 m/s. Additionally, even fewer weighting curves were similar for perturbed and unperturbed walking at the lowest walking speed.

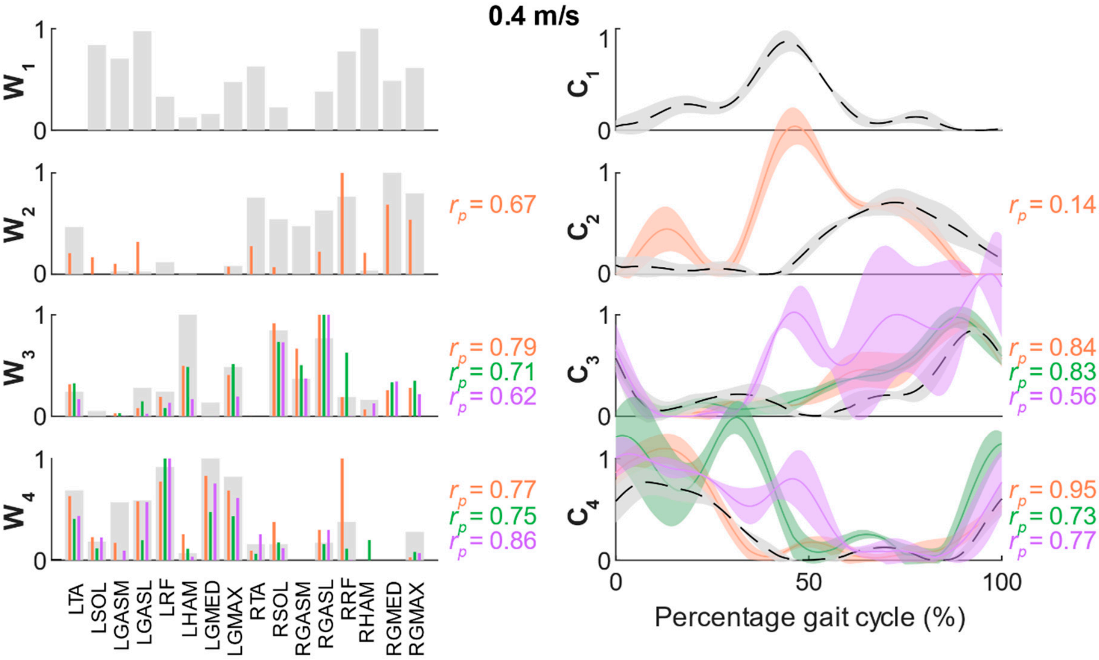


(**a**)

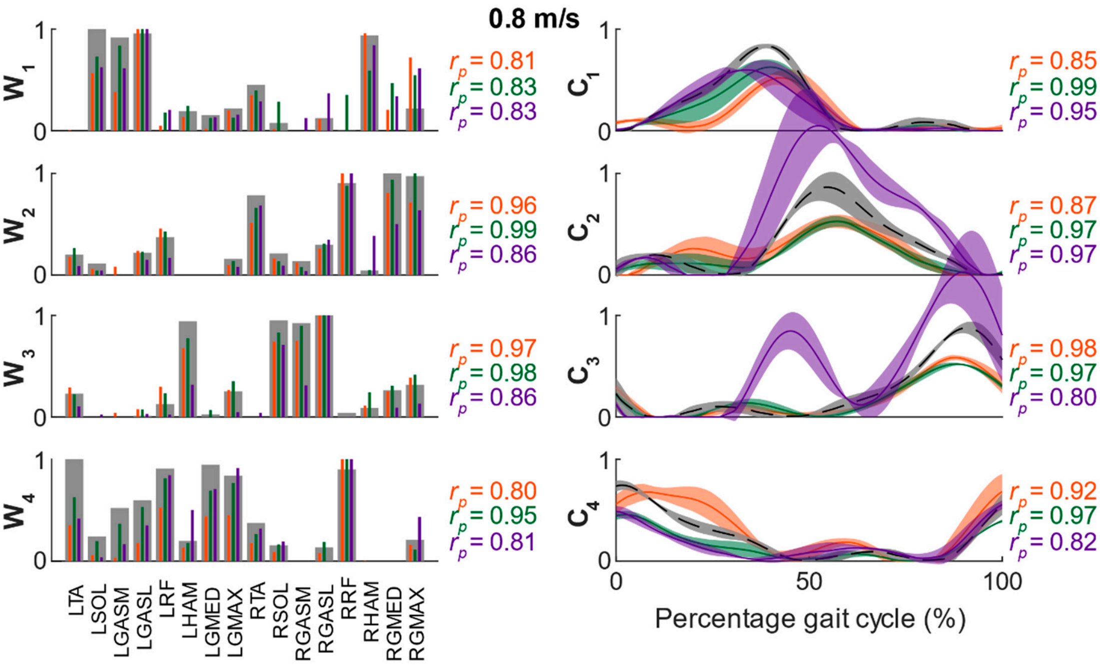


(**b**)

Figure 5. Muscle synergies and weighting curves for outward-directed perturbed walking and unperturbed walking at (**a**) the lowest and (**b**) the fastest walking speed for a representative subject. The colors indicate unperturbed walking (grey) and perturbed walking, with a perturbation onset of 0% (orange), 30% (green), or 60% (purple) of the stance phase of the gait cycle. (**left**) Muscle synergies for unperturbed (wide grey bars) and outward-directed perturbed walking for different perturbation onsets (narrow colored bars). Muscle synergies for perturbed walking were only shown when similar to the corresponding muscle synergy for unperturbed walking ($r_p \geq 0.623$). (**right**) Weighting curves for unperturbed (black striped line) and perturbed walking with different perturbation onsets (colored solid lines). Weighting curves are displayed as the mean value (lines) ± standard deviation (shaded area) of three repetitions.



(**a**)



(**b**)

Figure 6. Muscle synergies and weighting curves for inward-directed perturbed walking and unperturbed walking at (**a**) the lowest and (**b**) the fastest walking speed for a representative subject. The colors indicate unperturbed walking (grey) and perturbed walking, with a perturbation onset of 0% (orange), 30% (green), or 60% (purple) of the stance phase of the gait cycle. (**left**) Muscle synergies for unperturbed (wide grey bars) and inward-directed perturbed walking for different perturbation onsets (narrow colored bars). Muscle synergies for perturbed walking were only shown when similar to the corresponding muscle synergy for unperturbed walking ($r_p \geq 0.623$). (**right**) Weighting curves for unperturbed (black striped line) and perturbed walking with different perturbation onsets (colored solid lines). Weighting curves are displayed as the mean value (lines) $\pm$ standard deviation (shaded area) of three repetitions.

These observations were consistent with the results across subjects. Figures for all subjects and walking speeds can be found in the Supplementary Materials. For walking at 0.8 m/s, all four muscle synergies for perturbed walking were similar to the four muscle synergies for unperturbed walking, regardless of perturbation condition ($r_p \geq 0.623$) (Figure 7). Significantly less than four muscle synergies for perturbed walking were similar to the four muscle synergies for unperturbed walking at the lowest walking speed (inwards: $p = 0.04$ and $p = 0.023$ for perturbation onsets 0% and 60% of the stance phase of the gait cycle, respectively; outwards: $p = 0.009$, $p = 0.024$, and $p = 0.003$ for perturbation onsets 0%, 30%, and 60% of the stance phase of the gait cycle, respectively).

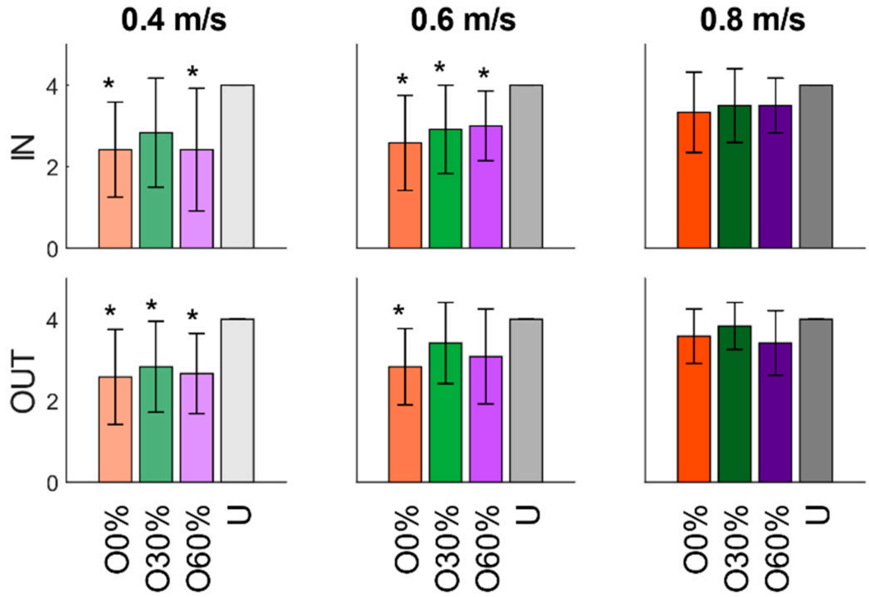


Figure 7. The number of perturbed muscle synergies similar to those for unperturbed walking, averaged across subjects. Asterisks (*) indicate significant differences in the number of perturbed synergies similar to unperturbed synergies ($p < 0.05$, one-way ANOVAs followed by Bonferroni post hoc tests). IN, OUT; inward-, outward-directed perturbation; O0%, O30%, O60%: perturbation onset of 0%, 30%, 60% of stance phase of gait cycle; U, unperturbed.

Further inspection of the synergies revealed which particular synergies were typically dissimilar to the corresponding unperturbed synergy (Figure 8). The summative count over all subjects of each individual synergy similar to the corresponding unperturbed synergy shows that mainly the push-off synergy was dissimilar at the lowest walking speed for both perturbation directions.

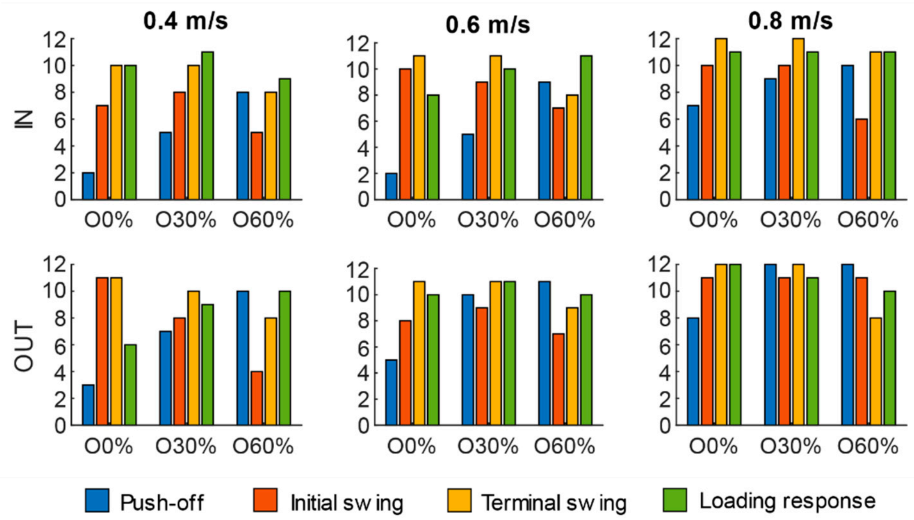


Figure 8. The summative count of the separate muscle synergies (push-off, initial swing, terminal swing, and loading response synergies) similar to the corresponding unperturbed synergy over all subjects. IN, OUT; inward-, outward-directed perturbation; O0%, O30%, O60%: perturbation onset of 0%, 30%, 60% of the stance phase of the gait cycle.

4. Discussion

The aim of this study was to investigate balance responses following perturbations in the mediolateral direction at different walking speeds. Previous studies investigating muscle synergies underlying dynamic balancing responses after inducing balance perturbations during gait have suggested that the same set of muscle synergies used during unperturbed walking is also used in perturbed walking [3,14]. However, these studies were performed at intermediate to high walking speeds (around 1.2 m/s). In line with these previous studies, our results for a high walking speed of 0.8 m/s demonstrated that four similar muscle synergies did indeed underly both unperturbed and perturbed gait. In our study, we also examined muscle synergies at substantially lower walking speeds (0.4 and 0.6 m/s). For these lower walking speeds, the muscle synergies for perturbed and unperturbed walking differed considerably. The number of muscle synergies for perturbed gait that were similar to those for unperturbed walking was significantly less than four for low walking speeds, with mainly the push-off synergy being substantially different.

4.1. Muscle Synergies

Outward-directed perturbations. Biomechanical outcomes that were previously reported and obtained in the same experiment showed that walking speed significantly affected the balance strategy selection for outward-directed perturbations [8]. At the highest walking speed, mainly the stepping response strategy, i.e., changing the location of the ensuing step, was used. On the other hand, for the lowest walking speed, the in-stance strategy, which modulates the center-of-pressure and the ground-reaction-forces under the leg in-stance, was predominantly used. In our previous study [9], we have shown that a substantial braking under the leg in-stance is needed to support the in-stance balancing strategy at the lowest walking speed. This braking is achieved by a substantial increase in plantar flexors activity, resulting in the observed changes in muscle synergies. Thus, for outward-directed perturbations, the observed decrease in number of shared synergies for the lowest walking speed was as expected. Due to the speed-dependent balance strategy selection, appropriate changes, predominantly in the underlying muscular activity of the leg in-stance, are required, which explains the reduced number of shared synergies.

Inward-directed perturbations. For inward-directed perturbations however, the biomechanical outcome measures have shown that the stepping strategy was used consistently at all walking speeds [8]. Accordingly, one would expect that underlying muscle synergies following inward perturbations would be substantially similar regardless of speed, which was not the case. The number of shared synergies underlying unperturbed and perturbed gait reduced with walking speed, for both inward- and outward-directed perturbations. Further inspection of the synergies revealed that mainly the push-off synergy was dissimilar at the lowest walking speed, regardless of perturbation direction. For inward-directed perturbations, the ensuing step following perturbations was substantially shorter at the lowest walking speed in comparison to the step length following perturbation at the highest walking speed [9]. The difference in the ensuing step length thus necessitates appropriate adjustment in the push-off power exerted by the ankle muscle of the leg in-stance. Consequently, the activity of the plantar flexors of the leg in-stance following the inward-directed perturbation at the lowest walking speed must be substantially reduced, resulting in the observed difference in muscle synergies.

4.2. Methodology and Limitations

Four muscle synergies were generally required to adequately reconstruct the original data with sufficiently high VAF values. Despite a few exceptional cases where five muscle synergies were needed, the number of muscle synergies was set at four to avoid noise being explained by additional muscle synergies [15]. Four muscle synergies were reported to be sufficient by the majority of similar studies [1,2,14,16,17].

Similarity between two muscle synergies was assessed by Pearson's correlation coefficient r_p. A pair of muscle synergies was considered as similar for $r_p \geq 0.623$, as in multiple other studies [1,3]. An additional analysis could have been performed to verify similarity. Muscle synergies of one data set could be used to reconstruct another data set in which muscle synergies were considered to be similar. Chvatal et al. [15] extracted functional muscle synergies from non-stepping data, and used them to reconstruct stepping data. The muscle synergies extracted from non-stepping data accounted for $85 \pm 3\%$ of the overall variability for the stepping data, while the variance-accounted-for was $93 \pm 1\%$ for the entire data set consisting of stepping and non-stepping data. Since muscle synergies for non-stepping data explain the stepping data sufficiently, the corresponding muscle synergies could be considered as similar. Such an approach would presumably not cause the present results to deviate, as it is expected that muscle synergies for perturbed and unperturbed walking do not differ more from each other than muscle synergies for stepping and non-stepping.

The muscle synergies and weighting curves correspond highly to those in the study by Severini et al. [14], in which four bilateral muscle synergies were also composed of combinations of unilateral muscle synergies. In general, the same muscles were included, and comparable activation patterns were shown. However, some main contributing muscles to the synergies found by Severini were not included in the present study. For instance, the vastus medialis was excluded in the present study, but contributed largely to Severini's early stance synergy. Similarly, the tensor fasciae latae was a main contributor to Severini's initial swing synergy. It is expected that including missing muscles would only add additional components to the existing synergies, while overall synergy structures would remain intact.

Applying perturbing pushes at the level of the pelvis during walking represents an ecologically valid perturbation modality that is related to real-life perturbations resulting from people bumping into each other in a crowd. Other researchers have used perturbing pushes at the pelvis extensively [6,18,19]. However, people also face other types of perturbations during walking, such as slips and trips. Although the balance responses to these perturbations may show some resemblance in terms of kinematics and kinetics to those in response to push perturbations [19], the underlying muscle synergies may differ. Therefore, the results of our study may not be generalizable across other types of perturbations.

5. Conclusions

The aim of this study was to investigate balance responses following perturbations in the mediolateral direction at different walking speeds. Stereotyped muscle synergies were used in unperturbed and perturbed walking at all tested speeds. However, while these synergies were similar at higher walking speeds, this was not the case at lower walking speeds.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app12042135/s1: Table of average and minimum VAF values (overall and per muscle) per subject and perturbation direction. Figures of the muscle synergies and weighting curves for inward- and outward-directed perturbations for all subjects, all walking speeds, and all perturbation onsets.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Slovenian National Ethics Committee.

Informed Consent Statement: Informed consent was obtained from all subjects involved.

Data Availability Statement: Data supporting reported results can be found in the Supplementary Materials.

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