Journal of NeuroEngineering and Rehabilitation

RESEARCH

Open Access

# Should you hold onto the treadmill handrails or not? Cortical evidence at different walking speeds

Monica Biggio[1], Costanza Iester[1], Davide Cattaneo[2,3], Simone Cutini[4], Ambra Bisio[5], Ludovico Pedullà[6], Alessandro Torchio[2], Marco Bove[5,7*] and Laura Bonzano[1,7]

## Abstract

**Background** Treadmill-based gait training is part of rehabilitation programs focused on walking abilities. The use of handrails embedded in treadmill systems is debated, and current literature only explores the issue from a behavioral perspective.

**Methods** We examined the cortical correlates of treadmill walking in healthy participants using functional near-infrared spectroscopy. We investigated whether the utilization of treadmill handrails at varying walking speeds could affect cortical activation associated with the task, and we evaluated potential differences in task-based functional connectivity across the various walking conditions.

**Results** Significant differences in cortical activation were found between the two walking speeds (3 and 5 km/h) in the unsupported condition; these differences were reduced when using the handrails. Specifically, cortical activation was significantly higher when the participants swung their arms freely while walking at a speed of 5 compared to 3 km/h in several Brodmann's Areas (BA): left BA10, BA3 and BA39, and right BA10, BA9, BA8, BA3, and BA40. No significant differences were found when participants were holding onto the handrails. A significant difference was found in the left BA40 between the two speeds, regardless of whether the participants were holding onto the handrails. Furthermore, at the higher speed and without the use of handrails, a wider pattern of task-based functional connectivity was observed, with significantly stronger connectivity between the left BA10 and BA40.

**Conclusions** We suggest that speed and handrails use play a role in walking cortical activity patterns, therefore they are key ingredients to take into account when planning a rehabilitation program.

**Keywords** Functional near-infrared spectroscopy, Haptic contact, Treadmill, Walking speed

*Correspondence:
Marco Bove
marco.bove@unige.it
[1]Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy
[2]IRCCS Fondazione Don Carlo Gnocchi, Milan, Italy
[3]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy
[4]Department of Developmental and Social Psychology, University of Padova, Padua, Italy
[5]Department of Experimental Medicine, Section of Human Physiology, University of Genoa, Viale Benedetto XV 3, 16132 Genoa, Italy
[6]Italian Multiple Sclerosis Foundation, Scientific Research Area, Genoa, Italy
[7]IRCCS Ospedale Policlinico San Martino, Genoa, Italy

## Background

Gait abnormalities during old age and as a result of neurological diseases are very debilitating and can increase the risk of falls [1], negatively affect independence and quality of life [2], and increase health care costs [3]. For this reason, walking abilities are often considered a primary focus for rehabilitation programs.

To create controlled setups in rehabilitation, several studies recommend treadmill-based gait training paradigms [4], and body weight support systems are allowed in order to minimize the delay in starting gait training for neurological patients and to increase safety without the use of walking aids [5]. The use of handrails embedded in treadmill systems helps to stabilize the body by increasing afferent somatosensory signals through haptic contact with the handrails [6–8].

Some controversial results have been reported in the literature on the use of treadmill handrails during rehabilitation. First of all, it should be considered that holding the hands onto the handrails of the treadmill might not imply a representative walking pattern, in terms of an ecological perspective, since it encourages bad posture and prevents the natural stride [9–11]. It has been reported that supporting on the handrails lightens the workload since it requires less muscular activation without resulting in substantial neuromuscular re-organization. In fact, it increases the base of support, resulting in greater stability, reduces uncertainty leading to a better balance, and improves ability to generate corrective forces to compensate for perturbations [12]. Also, handrail use during treadmill walking in a split-belt adaptation training reduced locomotor learning in healthy young subjects, suggesting that this balance support may ease, or alter the task demand [6].

It has been shown that the effect of gait rehabilitation can be improved by holding handrails [13], especially when participants used a firm rather than a light touch on the handrails [12]. It should be noted, however, that Bello and colleagues attributed the improvements seen during the rehabilitation of patients with Parkinson's disease to the belts used in combination with the treadmill, instead of the handrails themselves [14].

Nevertheless, all these studies explored the issue from a behavioral perspective. To our knowledge, the effects of holding handrails during treadmill walking have never been investigated with neuroimaging techniques.

In general, in order to propose effective rehabilitation paradigms, it is necessary to reach a better understanding of the mechanisms underlying the gait under various conditions [15]. Walking has long been regarded as predominantly automatic process, however functional magnetic resonance imaging (fMRI) studies based on motor imagery of gait have demonstrated a cortical control even during simple walking processes in healthy elderly, pathological subjects, as well as healthy young individuals [16–18]. The areas mainly involved in gait are the prefrontal cortex, supplementary motor, premotor and primary motor areas, sensorimotor areas; their activity has been found to be modulated by task demand [17, 19]. Bakker and colleagues asked their participants to (visually) imagine a normal gait or a precision gait over a narrower path, finding an increased cortical activity in cortical structures outside primary motor regions during the harder task, thus emphasizing greater cortical activity when an increased postural control is required [16].

Portable neuroimaging techniques, such as functional near-infrared spectroscopy (fNIRS), led to identify cortical activation patterns and locomotor networks involved in walking, providing new insight into cortical control of actual human locomotion [20–22]. In fact, this technique made it possible to study walking during its actual performance and to modulate the difficulty of the task [19], the somatosensory feedback from different peripheral stimuli [23], or to assess the difference between walking and running [24].

In this vein, we investigated the cortical correlates of treadmill walking by means of fNIRS in a group of healthy participants. We were interested in understanding whether the use of treadmill handrails, at different walking speeds, could modulate cortical activation related to the task. Specifically, we carried on an fNIRS study while walking on a treadmill at two different speeds (3 and 5 km/h - lower or equal with respect to the spontaneous walking speed of young healthy subjects [25]), with or without holding onto the handrails. Furthermore, one published study based on fMRI showed that individuals with faster gait speed have stronger resting-state functional connectivity (FC) within the frontoparietal control network, and that gait variability is correlated with between-network functional connectivity [26]. As a step forward, here using fNIRS we were able to assess possible differences in task-based FC among the task conditions during walking to better understand how task performance modulated the connectivity between cortical regions, providing a more comprehensive view of cortical function beyond isolated regional activity.

It has been reported that, during comfortable walking, lower extremity muscle activity had a strong correlation with cortical activation [27]. Therefore, we expected to find differences in cortical activation between the two walking speeds in the unsupported condition. Given that walking with handrails can lighten the workload of walking requiring less muscular activation [12], we hypothesized that this condition would be associated with a reduced brain resource demand and that the use of the handrails could mitigate the differences in cortical activity due to the walking speed.

## Materials and methods

### Participants

Twenty-four healthy, right-handed volunteers were included in this study (age = 27.0 ± 6.3 years, 13 females). None of them had a history of orthopedic or neurological disease and none had previously trained in treadmill walking or running. Those who regularly train on the treadmill were excluded from recruitment. All the participants were naïve to the specific purpose of the study and provided written informed consent to participate. The study was approved by the University Ethics Committee (CERA), Genoa, Italy (N. 2023/44). The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

### Experimental protocol

Participants were asked to perform different locomotor tasks on a treadmill (Technogym, Italy), and cortical activation elicited by the tasks was assessed through fNIRS. A box-car design was implemented, with 30 s of walking (differently depending on the specific task condition) and 30 s of rest in an upright position (Fig. 1).

Before the acquisition session, participants familiarized with the task - hence the speed and the holding conditions - that would follow by walking on the treadmill at 3 km/h and 5 km/h, both with and without holding onto the handrails. Then, task conditions were four: in two conditions, they walked with a spontaneous swing of the arms, and the speed of the treadmill was set at 3 km/h or at 5 km/h (V3_no-HOLD and V5_no-HOLD conditions, respectively); in the other two conditions, participants were asked to repeat the two conditions leaning on the handrails of the treadmill while walking at 3 km/h or at 5 km/h (V3_HOLD and V5_HOLD conditions, respectively). During the rest periods, participants remained in an upright position on the treadmill, with their arms along the body or with their hands on the handrails, according to the previous task condition (no-HOLD or HOLD, respectively). Notably, the rest period allowed the hemodynamic signal to return to baseline.

Each condition was repeated eight times, and the order of the conditions was randomized by a custom script created with OpenSesame version 3.2.7 [28], which played a *start* audio signal at the beginning of the task and a *stop* audio signal at the end of the 30 s walk. Moreover, using the PsychoPy backend [29] of OpenSesame, the

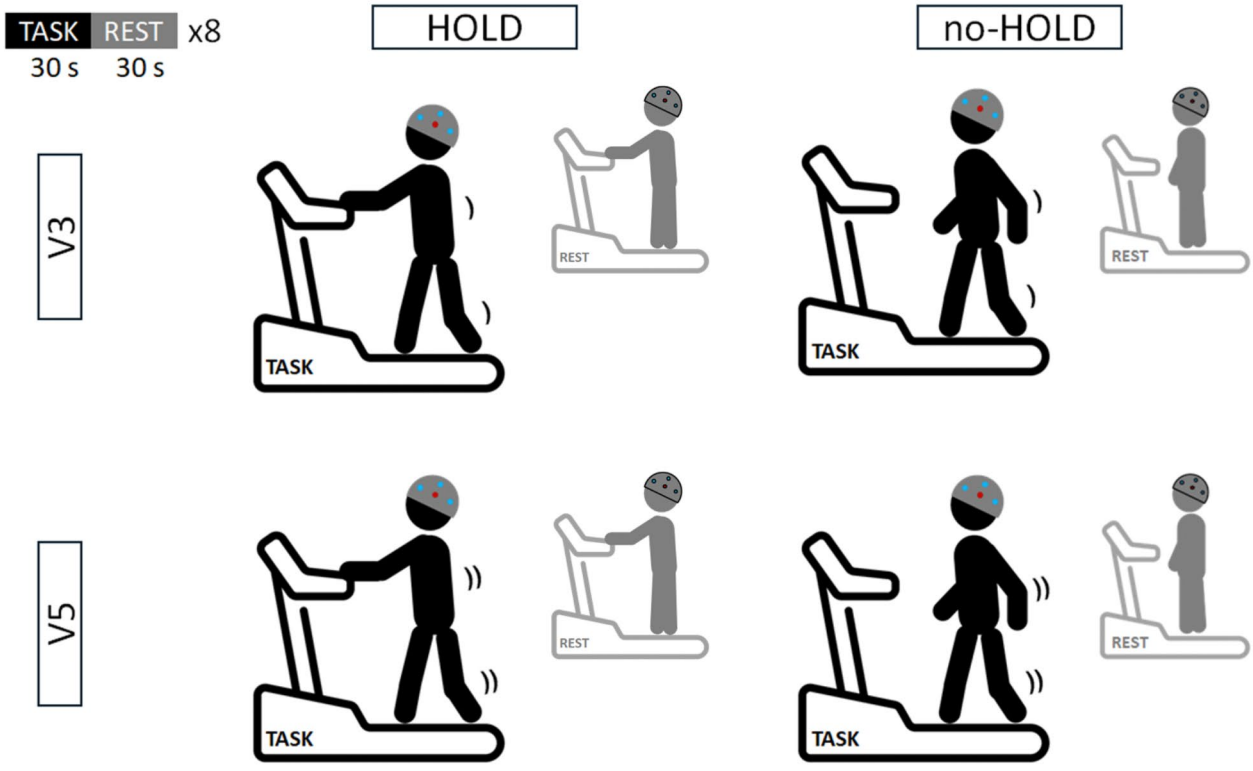


**Fig. 1** Experimental protocol. A box-car design was implemented, with 30 s of walking on a treadmill and 30 s of rest in an upright position. Walking was performed in four different randomized conditions: the speed of the treadmill was set at 3 km/h or at 5 km/h, and participants kept their hands on the handrails (V3_HOLD and V5_HOLD respectively) or walked with a spontaneous swing of the arms (V3_no-HOLD and V5_no-HOLD respectively). During the rest period, participants kept their arms along the body or their hands on the handrails, depending on the related task condition. Each trial was repeated eight times

Lab Streaming Layer protocol [30] was implemented in order to indicate the beginning of each block over the fNIRS recording and enable off-line analysis of the different conditions, separately.

At the end of each condition, participants were asked to rate their perceived levels of fatigue and task difficulty using a Visual Analog Scale (VAS). The VAS scale ranged from 0 to 10, where 0 indicated "no fatigue" or "no difficulty" and 10 represented "extreme fatigue" or "extreme difficulty". This approach allowed us to gather subjective feedback on the exertion and complexity associated with each condition, providing additional insights into participants' experiences during the task.

The entire experimental protocol lasted 32 min.

#### fNIRS data acquisition

Cortical hemodynamic activity was estimated by means of fNIRS, using a portable, multichannel NIRS system (NIRSport 2, NIRx Medical Technologies, Berlin, Germany) to allow for the calculation of changes in the concentration of oxyhemoglobin (HbO) and deoxyhemoglobin (HbR). Pairs of sources and detectors operating at two continuous wavelengths of near-infrared light (760 nm and 850 nm) generated measurement channels. All single-tip optodes were placed on a soft black tissue cap (EasyCap, Germany) worn by the participant, with different cap sizes used according to own head circumference. Sixteen sources and 16 detectors were arranged to form 44 standard channels (3 cm) covering the prefrontal, frontal, sensorimotor and parietal areas (Table 1). In addition, 8 short-separation (SS) channels (8 mm) were used to isolate and remove the extracerebral signals, which include blood pressure weaves, Mayer waves, respiration and cardiac cycles and can be seen as the noise in the signal of the long channels [31]. The sampling frequency was set at 8.7 Hz.

#### fNIRS data analysis

For each participant, the fNIRS signal was pre-processed using MATLAB version R2023b (MathWorks, MA, USA) through custom scripts including some of the Homer3 NIRS processing package functions [32]. Channels with low signal-to-noise ratio were discarded (SNR < 2). Then, the intensity data of the remaining channels were converted to optical density changes. Motion artifacts were identified by applying the Homer3 function *hmrR_MotionArtifactByChannel* on changes in optical density data (AMPthresh = 0.5; STDEVthresh = 12; tMotion = 0.5; and tMask = 1) [33–36] and corrected by applying a combination [36, 37] of spline ($p = 0.99$) [38, 39] and wavelet (iqr = 0.5) [40] motion correction techniques. A band-pass filter (0.01–3 Hz) was applied to remove slow drifts, and high frequencies contribution. Then, with the *hmrR_StimRejection* function, we assessed the absence of trials containing residual motion artifacts before the calculation of the hemodynamic response function (HRF). An age-dependent differential pathlength factor was computed for each participant [41], and then the HbO and HbR concentration changes were computed through the modified Beer-Lambert law [42].

#### Statistical analysis

To calculate the mean HRF for each condition, participant, and channel, a General Linear Model (GLM) was applied. Iterative weighted least squares were used to solve the GLM [43]. A set of a consecutive sequence of gaussian functions with a spacing and standard deviation of 2 s was used as temporal basis functions for HRF [44–46]. The interval for the block average was set from − 2 to 45 s from stimulus onset. Baseline-corrected averaging was assessed considering the mean value of the interval between − 2 and 0 s with respect to the onset of each walking block as baseline. As an additional regressor in the GLM, the most correlated SS channel was added. The SS channel regression led to the reduction of the physiological noise.

HbO and HbR concentration changes of channels belonging to the same hemisphere and Brodmann's Area (BA) were averaged separately obtaining the signal in eighteen regions of interest (BA3, BA4, BA6, BA7, BA8, BA9, BA10, BA39, and BA40 for both the left and right hemispheres) [47, 48].

The statistical analyses employed an average metric of the hemodynamic responses in the range of 5 to 20 s after stimulus onset for each participant, condition, and BA to avoid gait initiation and to measure activation at steady-state speed.

In order to identify the BAs that were significantly activated during walking in the different conditions, after testing for normality with the Shapiro-Wilk test, the one-sample Wilcoxon signed-rank test was performed to compare the changes in HbO concentration against zero, which represents no change in concentration given that HbO concentration changes were corrected for baseline [44]. Using this procedure, significant HbO concentration changes, indicative of activation elicited by the task, were evaluated for each BA and condition. Bonferroni correction for multiple comparisons was applied considering 18 BAs (Bonferroni adjusted p value threshold: 0.05/18 = 0.0028). The same analysis was conducted for HbR concentration changes.

Then, Friedman's ANOVA was used to analyze HbO concentration changes in the BAs which were found to be active in at least one condition in the group results, across the different within-subjects conditions. To examine where the differences actually occurred, post-hoc analysis with Durbin-Conover pairwise comparisons (V5_no-HOLD vs. V3_no-HOLD, V5_HOLD vs.

**Table 1** List of standard fNIRS channels and their correspondence to Brodmann's areas: source and detector positions according to the standard 10–10 EEG system; channel MNI coordinates (mm), anatomical description, and number of underlying BA

| Ch | Source | Detector | X | Y | Z | Laterality | Lobe | Anatomical location | BA |
|---|---|---|---|---|---|---|---|---|---|
| 1 | CP1 | C1 | -27 | -36 | 71 | Left | Parietal | Postcentral Gyrus | 3 |
| 2 | C3 | C1 | -42 | -20 | 62 | Left | Frontal | Precentral Gyrus | 4 |
| 3 | FC1 | FC3 | -38 | 12 | 55 | Left | Frontal | Middle Frontal Gyrus | 6 |
| 4 | FC1 | FCz | -13 | 12 | 67 | Left | Frontal | Superior Frontal Gyrus | 6 |
| 5 | FC1 | C1 | -26 | -5 | 68 | Left | Frontal | Superior Frontal Gyrus | 6 |
| 6 | C3 | FC3 | -50 | -3 | 50 | Left | Frontal | Precentral Gyrus | 6 |
| 7 | Cz | C1 | -17 | -20 | 74 | Left | Frontal | Precentral Gyrus | 6 |
| 8 | CP1 | P1 | -24 | -62 | 62 | Left | Parietal | Superior Parietal Lobule | 7 |
| 9 | CP1 | CPz | -16 | -50 | 72 | Left | Parietal | Postcentral Gyrus | 7 |
| 10 | P3 | P1 | -32 | -73 | 47 | Left | Parietal | Superior Parietal Lobule | 7 |
| 11 | Pz | P1 | -13 | -73 | 56 | Left | Parietal | Superior Parietal Lobule | 7 |
| 12 | FC1 | F1 | -23 | 26 | 56 | Left | Frontal | Superior Frontal Gyrus | 8 |
| 13 | F3 | F1 | -31 | 39 | 41 | Left | Frontal | Middle Frontal Gyrus | 9 |
| 14 | F3 | FC3 | -45 | 25 | 41 | Left | Frontal | Middle Frontal Gyrus | 9 |
| 15 | Fz | F1 | -9 | 41 | 50 | Left | Frontal | Superior Frontal Gyrus | 9 |
| 16 | Fpz | Fp1 | -12 | 67 | 0 | Left | Frontal | Medial Frontal Gyrus | 10 |
| 17 | AF3 | Fp1 | -24 | 63 | 9 | Left | Frontal | Middle Frontal Gyrus | 10 |
| 18 | AF3 | AFz | -12 | 62 | 23 | Left | Frontal | Superior Frontal Gyrus | 10 |
| 19 | AF3 | F1 | -23 | 52 | 32 | Left | Frontal | Superior Frontal Gyrus | 10 |
| 20 | P3 | CP3 | -46 | -61 | 46 | Left | Parietal | Inferior Parietal Lobule | 39 |
| 21 | C3 | CP3 | -52 | -34 | 52 | Left | Parietal | Postcentral Gyrus | 40 |
| 22 | CP1 | CP3 | -39 | -48 | 60 | Left | Parietal | Inferior Parietal Lobule | 40 |
| 23 | CP2 | C2 | 27 | -35 | 71 | Right | Parietal | Postcentral Gyrus | 3 |
| 24 | C4 | C2 | 42 | -21 | 62 | Right | Frontal | Precentral Gyrus | 4 |
| 25 | Cz | C2 | 17 | -21 | 75 | Right | Frontal | Precentral Gyrus | 6 |
| 26 | FC2 | FCz | 14 | 13 | 66 | Right | Frontal | Superior Frontal Gyrus | 6 |
| 27 | FC2 | FC4 | 39 | 12 | 54 | Right | Frontal | Middle Frontal Gyrus | 6 |
| 28 | FC2 | C2 | 27 | -4 | 68 | Right | Frontal | Superior Frontal Gyrus | 6 |
| 29 | C4 | FC4 | 52 | -4 | 48 | Right | Frontal | Precentral Gyrus | 6 |
| 30 | Pz | P2 | 15 | -73 | 57 | Right | Parietal | Superior Parietal Lobule | 7 |
| 31 | P4 | P2 | 33 | -74 | 48 | Right | Parietal | Superior Parietal Lobule | 7 |
| 32 | CP2 | CPz | 17 | -50 | 73 | Right | Parietal | Postcentral Gyrus | 7 |
| 33 | CP2 | P2 | 25 | -62 | 63 | Right | Parietal | Superior Parietal Lobule | 7 |
| 34 | FC2 | F2 | 24 | 26 | 55 | Right | Frontal | Superior Frontal Gyrus | 8 |
| 35 | Fz | F2 | 10 | 41 | 50 | Right | Frontal | Superior Frontal Gyrus | 9 |
| 36 | F4 | F2 | 30 | 40 | 41 | Right | Frontal | Middle Frontal Gyrus | 9 |
| 37 | F4 | FC4 | 44 | 25 | 40 | Right | Frontal | Middle Frontal Gyrus | 9 |
| 38 | Fpz | Fp2 | 13 | 67 | 0 | Right | Frontal | Medial Frontal Gyrus | 10 |
| 39 | AF4 | AFz | 13 | 61 | 24 | Right | Frontal | Superior Frontal Gyrus | 10 |
| 40 | AF4 | Fp2 | 25 | 63 | 9 | Right | Frontal | Middle Frontal Gyrus | 10 |
| 41 | AF4 | F2 | 22 | 52 | 33 | Right | Frontal | Superior Frontal Gyrus | 10 |
| 42 | P4 | CP4 | 46 | -62 | 47 | Right | Parietal | Inferior Parietal Lobule | 39 |
| 43 | CP2 | CP4 | 39 | -49 | 60 | Right | Parietal | Postcentral Gyrus | 40 |
| 44 | C4 | CP4 | 52 | -35 | 52 | Right | Parietal | Postcentral Gyrus | 40 |

V3_HOLD, V5_no-HOLD vs. V5_HOLD, V3_no-HOLD vs. V3_HOLD) was conducted with a Bonferroni correction for multiple comparisons, resulting in a significance level set at $p < 0.0125$. As a measure of effect size, Kendall's W and rank correlation coefficient r were reported for Friedman's ANOVA and Durbin-Conover test, respectively.

Statistical analysis was conducted with Jamovi (version 2.3.21.0 [Computer Software] retrieved from https://www.jamovi.org). Data are reported as median (interquartile range).

In addition, correlation matrices were built based on Spearman's pairwise correlation coefficients between all the analyzed BAs, for each task condition separately, to

calculate BA-to-BA task-based FC. Then, the resulting four FC matrices per subject were compared at a node-wise level; for each node, we converted the correlation coefficients using the Fisher's r to Z transformation [49] and we tested whether the correlation coefficients were statistically different across the various conditions (with the same approach described in [50]). Since this analysis involved all the possible combinations between each BA, the resulting statistical values of the comparisons between the correlation coefficients were corrected with Bonferroni method (Bonferroni adjusted p value threshold: 0.05/153 = 0.00033).

## Results

### Visual analog scale

Overall, participants did not report the task as difficult or fatiguing. VAS scores for both fatigue and task difficulty remained low across all conditions (Difficulty median scores with interquartile ranges (IQR): V5_HOLD = 0[0–1]; V5_no-HOLD = 0[0–1]; V3_HOLD = 0[0–0] and V3_no-HOLD = 0[0–0]; Effort median scores with interquartile ranges (IQR): V5_HOLD = 1[0–1]; V5_no-HOLD = 1[0–1]; V3_HOLD = 0[0–1] and V3_no-HOLD = 0[0-0.5]).

### Cortical activity

Figure 2; Table 2 show the BAs which were found to be significantly active in the different task conditions ($p < 0.0028$). Specifically, the task in the V5_no-HOLD condition elicited activation in the left BA10, BA8, BA6, BA3, BA39 and BA40 and the right BA10, BA9, BA8 and BA40. In the V3_no-HOLD condition, the right BA10, BA9 and BA6 were significantly active. When participants were holding onto the treadmill handrails, walking at 5 km/h (V5_HOLD) induced significant activation of the left BA10 and BA40 and the right BA10, BA9 and BA3; whilst walking at 3 km/h (V3_HOLD) induced significantly activation of the left BA3 and the right BA10.

Then, differences in cortical activation among the different task conditions were assessed (Table 3). There was a statistically significant difference in HbO concentration changes depending on task condition in the following BAs: left BA10 ($\chi^2$ [3] = 10.1, $p = 0.018$, W = 0.14), BA8 ($\chi^2$ [3] = 9.15, $p = 0.027$, W = 0.13), BA3 ($\chi^2$ [3] = 9.75, $p = 0.021$, W = 0.14), BA39 ($\chi^2$ [3] = 14.8, $p = 0.002$, W = 0.21) and BA40 ($\chi^2$ [3] = 21.8, $p < 0.001$, W = 0.30), and right BA10 ($\chi^2$ [3] = 9.65, $p = 0.022$, W = 0.13), BA9 ($\chi^2$ [3] = 13.1, $p = 0.004$, W = 0.18), BA8 ($\chi^2$ [3] = 11.8, $p = 0.008$, W = 0.16), BA3 ($\chi^2$ [3] = 10.8, $p = 0.013$, W = 0.15) and BA40 ($\chi^2$ [3] = 11.4, $p = 0.010$, W = 0.16).

As illustrated in Fig. 3, post-hoc analysis showed that HbO concentration change in the left BA10 in the no-HOLD condition was significantly higher when speed was 5 km/h with respect to 3 km/h (V5_no-HOLD vs. V3_no-HOLD: 181.0 nM (182.4) vs. 55.7 nM (271.4), $p = 0.011$, $r = 0.53$); no difference was found either between the two speeds in the HOLD condition or between the two holding conditions when considering the two speeds separately. Analogous results were found in the left BA3 (V5_no-HOLD vs. V3_no-HOLD: 184.0 nM (223.5) vs. 51.1 nM (149.8), $p = 0.002$, $r = 0.65$) and BA39 (V5_no-HOLD vs. V3_no-HOLD: 86.9 nM (136.7) vs. 27.9 nM (142.3), $p < 0.001$, $r = 0.75$). Furthermore, HbO concentration change in the left BA40 in the no-HOLD condition was significantly higher when speed was 5 km/h with respect to 3 km/h (V5_no-HOLD vs. V3_no-HOLD: 122.0 nM (249.0) vs. 90.1 nM (159.2), $p = 0.002$, $r = 0.64$); also, a similar significant difference between the two speeds was found in the HOLD condition (V5_HOLD vs. V3_HOLD: 142.0 nM (157.8) vs. 23.2 nM (131.1), $p < 0.001$, $r = 0.91$). No difference between the two holding conditions was found when considering the two speeds separately.

Considering HbO concentration changes in the left BA8, no statistical comparison survived.

From the analysis of HbO concentration changes in the right hemisphere, cortical activation in the no-HOLD condition was significantly higher when speed was 5 km/h with respect to 3 km/h in the following BAs: right BA10 (V5_no-HOLD vs. V3_no-HOLD: 210.0 nM (205) vs. 89.0 nM (229.7), $p = 0.009$, $r = 0.55$), BA9 (V5_no-HOLD vs. V3_no-HOLD: 143.0 nM (178.8) vs. 88.2 nM (80.8), $p = 0.001$, $r = 0.69$), BA8 (V5_no-HOLD vs. V3_no-HOLD: 148.0 nM (270.3) vs. 79.1 nM (159.8), $p < 0.001$, $r = 0.71$), BA3 (V5_no-HOLD vs. V3_no-HOLD: 119.0 nM (229.4) vs. 37.4 nM (160.4), $p = 0.008$, $r = 0.56$), BA40 (V5_no-HOLD vs. V3_no-HOLD: 132.0 nM (150.1) vs. 42.6 nM (114.1), $p < 0.001$, $r = 0.71$); no differences in these BAs were found either between the two speeds in the HOLD condition or between the two holding conditions was found when considering the two speeds separately.

When assessing the HbR concentration changes, no BA survived the Wilcoxon signed-rank test against zero with Bonferroni correction, so no further statistical analyses were performed on these data.

### Task-based functional connectivity

We assessed pairwise task-related FC considering all the analyzed BAs. As can be seen in Fig. 4, in general, higher FC was found in the no-HOLD conditions compared to the HOLD conditions.

Spearman's correlation coefficient for each couple of BAs is reported in a color-coded scale ranging from blue to yellow, only for those BA-to-BA correlations resulted to be statistically significant. White square indicates autocorrelation, grey square indicates non-significant correlation between the two BAs.

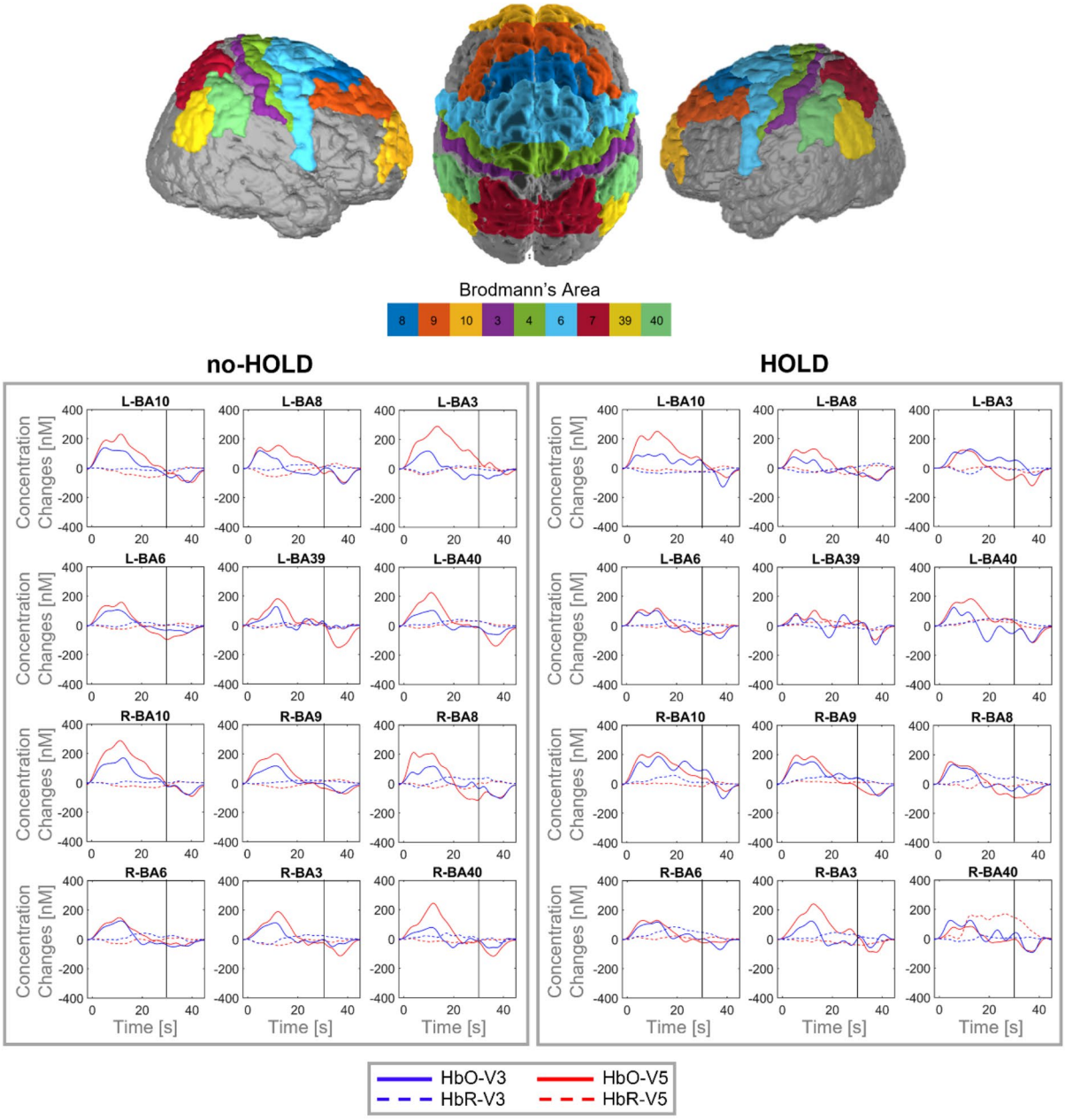


**Fig. 2** Brodmann's areas which resulted to be significantly activated by walking in at least one task condition, displayed on a representative brain template, and their hemodynamic responses. The solid and dashed lines represent the concentrations of HbO and HbR, respectively, in the different task conditions: no-HOLD (left panel) and HOLD (right panel) at 3 km/h (blue lines) and 5 km/h (red lines). Vertical bars represent the end of the task block

In details, in the V5_no-HOLD condition strong correlations were found at the intra-hemispheric level, in the left hemisphere more than in the right hemisphere, identifying a frontoparietal network (L-BA10, L-BA9, L-BA8 ↔ L-BA7, L-BA39, L-BA40) and a sensorimotor network (L-BA3 ↔ L-BA6) in the left hemisphere, and two networks, i.e., sensorimotor (R-BA3 ↔ R-BA4, R-BA6) and parietal-associative (R-BA7 ↔ R-BA39) in the right hemisphere. Moderate inter-hemispheric FC was found among fronto-parietal cortical areas.

In the V5_HOLD condition, a spread and moderate intra-hemispheric FC was observed in the left hemisphere; in the right hemisphere, FC was reduced and revealed a frontal network (R-BA10, R-BA9, R-BA8 ↔ R-BA6) and a parietal network (R-BA7 ↔ R-BA39). At the inter-hemispheric level, FC was similar to the corresponding no-HOLD condition.

**Table 2** Left (L) and right (R) Brodmann's areas significantly active in the different task conditions (one-sample Wilcoxon signed-rank test versus 0, Bonferroni correction resulting in $p < 0.0028$). * indicates statistical significance

| | V5_no-HOLD | | V3_no-HOLD | | V5_HOLD | | V3_HOLD | |
|---|---|---|---|---|---|---|---|---|
| | W | p | W | p | W | p | W | p |
| L-BA10 | 279 | < 0.001* | 235 | 0.014 | 272 | < 0.001* | 231 | 0.019 |
| L-BA9 | 242 | 0.007 | 200 | 0.160 | 239 | 0.010 | 201 | 0.152 |
| L-BA8 | 278 | < 0.001* | 166 | 0.663 | 252 | 0.003 | 211 | 0.084 |
| L-BA6 | 273 | < 0 0.001* | 249 | 0.004 | 240 | 0.009 | 207 | 0.107 |
| L-BA4 | 229 | 0.023 | 145 | 0.900 | 235 | 0.014 | 212 | 0.079 |
| L-BA3 | 279 | < 0.001* | 200 | 0.160 | 248 | 0.004 | 270 | < 0.001* |
| L-BA7 | 214 | 0.069 | 174 | 0.509 | 196 | 0.197 | 102 | 0.178 |
| L-BA39 | 266 | < 0.001* | 200 | 0.160 | 233 | 0.018 | 150 | 1.000 |
| L-BA40 | 258 | 0.001* | 214 | 0.069 | 273 | < 0.001* | 192 | 0.241 |
| R-BA10 | 296 | < 0.001* | 278 | < 0.001* | 299 | < 0.001* | 267 | < 0.001* |
| R-BA9 | 283 | < 0.001* | 273 | < 0.001* | 296 | < 0.001* | 245 | 0.005 |
| R-BA8 | 253 | 0.002* | 227 | 0.027 | 241 | 0.008 | 226 | 0.029 |
| R-BA6 | 245 | 0.005 | 254 | 0.002* | 247 | 0.004 | 238 | 0.011 |
| R-BA4 | 200 | 0.160 | 190 | 0.264 | 244 | 0.006 | 180 | 0.406 |
| R-BA3 | 251 | 0.003 | 212 | 0.079 | 276 | < 0.001* | 212 | 0.079 |
| R-BA7 | 222 | 0.039 | 169 | 0.603 | 223 | 0.037 | 165 | 0.684 |
| R-BA39 | 205 | 0.121 | 174 | 0.509 | 205 | 0.121 | 137 | 0.726 |
| R-BA40 | 270 | < 0.001* | 205 | 0.121 | 221 | 0.042 | 223 | 0.037 |

**Table 3** Comparisons of the active Brodmann's areas across the different task conditions (Friedman's ANOVA with significance level at $p = 0.05$ and Durbin-Conover pairwise comparisons with Bonferroni-adjusted $p = 0.0125$). * indicates statistical significance. n.s. indicates a non-significant contrast, consequently to the result of Friedman's ANOVA not reaching statistical significance

| BA | $\chi^2$ (3) | *p* | V5_no-HOLD vs. V3_no-HOLD | V5_HOLD vs. V3_HOLD | V5_no-HOLD vs. V5_HOLD | V3_no-HOLD vs. V3_HOLD |
|---|---|---|---|---|---|---|
| L-BA10 | 10.1 | 0.018* | **0.011*** | 0.048 | 0.445 | 0.860 |
| L-BA8 | 9.15 | 0.027* | 0.016 | 0.050 | 0.726 | 0.907 |
| L-BA6 | 5.15 | 0.161 | n.s. | n.s. | n.s. | n.s. |
| L-BA3 | 9.75 | 0.021* | **0.002*** | 0.907 | 0.293 | 0.029 |
| L-BA39 | 14.8 | 0.002* | **< 0.001*** | 0.053 | 0.181 | 0.714 |
| L-BA40 | 21.8 | < 0.001* | **0.002*** | **< 0.001*** | 0.696 | 0.362 |
| R-BA10 | 9.65 | 0.022* | **0.009*** | 0.082 | 0.907 | 0.413 |
| R-BA9 | 13.1 | 0.004* | **0.001*** | 0.057 | 0.630 | 0.336 |
| R-BA8 | 11.8 | 0.008* | **< 0.001*** | 0.285 | 0.124 | 0.405 |
| R-BA6 | 5.25 | 0.154 | n.s. | n.s. | n.s. | n.s. |
| R-BA3 | 10.8 | 0.013* | **0.008*** | 0.047 | 0.813 | 0.345 |
| R-BA40 | 11.4 | 0.010* | **< 0.001*** | 0.553 | 0.046 | 0.406 |

Examining BA-to-BA FC in the V3_no-HOLD condition, FC was found to be reduced than in the V5_no-HOLD condition, with moderate correlation between motor cortical areas (L-BA4 ↔ L-BA6). In the right hemisphere, fronto-parietal cortical areas showed strong or moderate correlation (R-BA9 ↔ R-BA3, R-BA4, R-BA6, R-BA7, R-BA39, R-BA40). In contrast, a comparable analysis of FC when participants were walking at 3 km/h holding onto the treadmill handrails (V3_HOLD) did not identify any connectivity in the prefrontal and parietal cortical areas, but a cluster of FC was identified in the sensorimotor cortical areas (L-BA4 ↔ L-BA3, L-BA6; R-BA3 ↔ R-BA4). Considering inter-hemispheric FC, one small cluster of strong FC was found in the prefrontal cortical areas (L-BA10 ↔ R-BA10). In addition, a moderate FC was observed among sensorimotor areas of the two hemispheres.

When comparing the different FC patterns observed during the different conditions, a visual inspection strongly suggested an influence dictated by task condition. In particular, there was a striking difference between the FC pattern observed in the V5_no-HOLD condition vs. the V3_HOLD condition. Indeed, among all the statistical comparisons of the different FC matrices across the conditions, only the node between L-BA10 and L-BA40 survived the Bonferroni correction (Z score = 3.49, $p = 0.00023$). Crucially, the correlation value between L-BA10 and L-BA40 was close to zero in the V3_HOLD condition, whereas it was extremely high ($r = 0.80$) in the V5_no-HOLD condition.

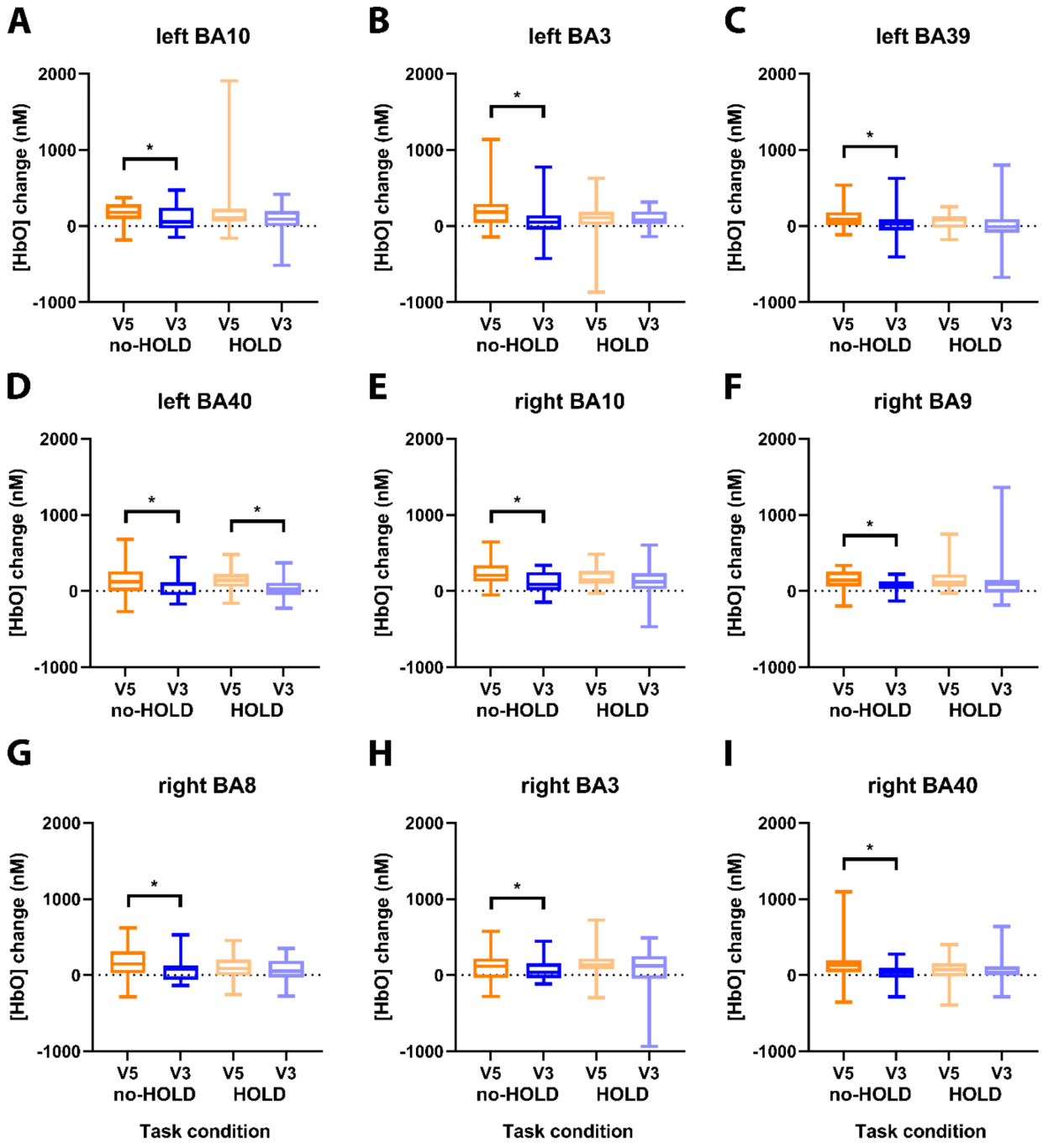


**Fig. 3** Changes in Oxy-hemoglobin concentration ([HbO]) in the different task conditions, in the Brodmann's areas showing statistically significant differences across conditions. The vertical bars represent the minimum and maximum values, the box illustrates the interquartile range, and the horizontal line inside the box indicates the median. * indicates statistical significance

## Discussion

The use of handrails in treadmill systems is a debated topic in the rehabilitation field, though so far it has only been explored from a behavioral perspective. We tried to give new hints in this framework, by conducting an fNIRS study in healthy volunteers walking on the treadmill. We found significant differences in cortical activation between two walking speeds (3–5 km/h) in the unsupported condition (not holding onto the handrails); the use of handrails (holding onto the handrails) canceled these differences, except for the left BA 40.

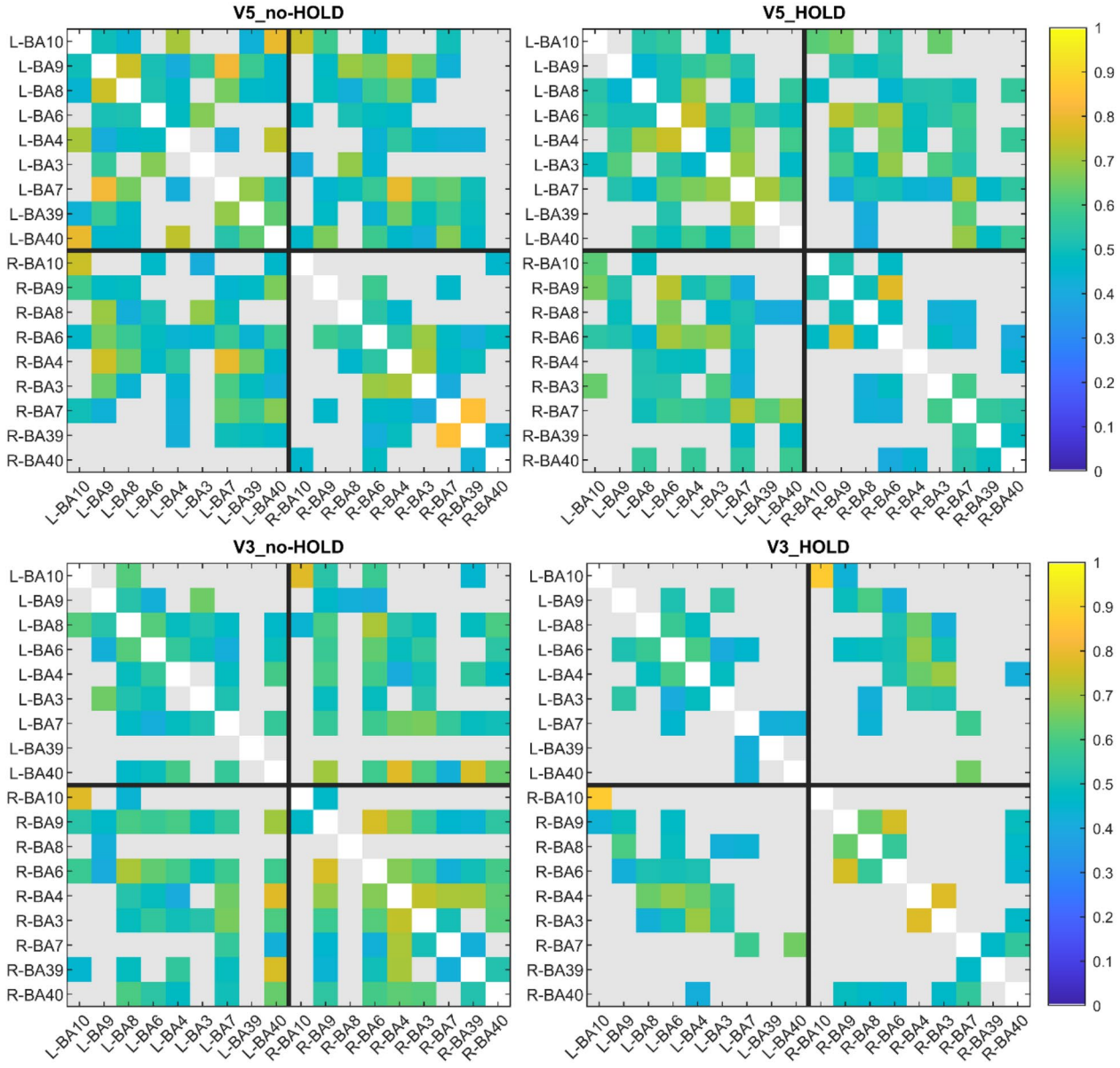


**Fig. 4** Correlation matrices representing pairwise BA-to-BA task-based functional connectivity

Generally, the premotor, sensorimotor, and associative areas are the main cortical regions involved during walking, working together in a complex system to regulate gait and maintain balance [51–54].

In this study we found a large network including prefrontal and parietal cortical areas. Interestingly, the pattern of activation was different across the several conditions under investigation. By a contrast analysis, we compared the cortical activations at the speed of 5 km/h between the HOLD and no-HOLD conditions, and the cortical activations at the speed of 3 km/h between the same conditions. We observed no significant differences between the two holding conditions, considering the two speeds separately. We could interpret these results in terms of similar cortical activation patterns, at both high and low speeds, when holding or not holding onto the treadmill handrails. Although the contrast analysis showed no significant results, we could observe that the cortical activation at the speed of 3 km/h in the two holding conditions had a common prefrontal cortical area (right BA10), but the activation pattern was slightly larger in the no-HOLD condition. This difference between the two holding conditions was exacerbated by speed: a wider activation of fronto-parietal areas was observed at the higher speed (5 km/h) in the unsupported compared to the supported condition. In

this regard, the comparison between the cortical activations at the two different speeds (3 km/h vs. 5 km/h) in the unsupported condition confirmed this observation. Specifically, cortical activation was significantly higher when the participants walked with a spontaneous swing of the arms at a speed of 5 km/h compared to 3 km/h in the following Brodmann's areas: left BA10, BA3, and BA39, and right BA10, BA9, BA8, BA3, and BA40. In the same cortical areas, no significant differences were found when participants were holding onto the handrails. These findings indicate that the more the speed increases, the more the cortical activation increases, and different areas are activated between the two conditions. Previously, it has been shown that the prefrontal and premotor cortices are involved in adapting to locomotor speed on the treadmill [24]. Further, the change in the left prefrontal cortex activation is associated with walking speed, being influenced by the participant's gait capacity at high walking speed [55].

However, when we compared the cortical activations in the two speeds in the supported condition only one difference remained, consisting in a higher activation of the left BA40 at the higher speed. The left inferior parietal cortex (left BA40) is involved in the cortical frontoparietal attention network [56].

The negligible modulation induced by speed in the supported condition is related to a generally reduced activity pattern, probably because keeping the hands on the treadmill handrails facilitates the task, regardless of speed. In the supported condition, the cortical activation pattern at the higher speed closely resembles that at the lower speed, with no significant differences between the speed conditions. Crucially, the reduced activation of the fronto-parietal areas in the supported condition contrasts with the unsupported condition, where those areas were clearly recruited at the higher speed. In the control of voluntary movement, these areas are related to attention and task complexity. During walking, the broader activation of the supplementary motor and premotor regions have been found to be related to variability of gait parameters [57]. This involvement could be regarded as indirect evidence that walking requires increased attention for the effort to match the target speed by modulating the gait parameters. Cortical activity is proportional to the difficulty in controlling dynamic balance rather than to the need to walk fast. This would also have implications for the use of endurance training methods, which should be performed without hand support, when possible, if more areas need to be recruited.

Furthermore, at higher speed and without the use of handrails, a wider pattern of task-based functional connectivity was observed, with significantly stronger connectivity between the left BA10 and left BA40. The former could have a role when increased cognitive control is required during walking at higher speed and without support, as evidenced in usual walking in patients with Parkinson's disease who need cognitive resources even in simple tasks [58]. The stronger connectivity between the left BA10 and the left BA40 supports the idea that walking requires considerable cognitive input, enriched by information from associative areas. Indeed, it has been shown that gait and cognitive activity share a frontoparietal network, and gait speed is significantly linked to functional connectivity within the frontoparietal network [26].

We could hypothesize that the higher activity elicited by treadmill walking in fronto-parietal areas in the V5_no-HOLD condition and the associated stronger frontoparietal connectivity in the left hemisphere are fundamental for inducing neuroplasticity phenomena. This is supported by previous studies indicating that higher neural activation is associated with early plasticity related to motor learning [59], and greater task complexity could be beneficial in inducing motor plasticity in younger adults [59–61]. Indeed, non-fatiguing conventional locomotor exercise decreases intracortical inhibition and/or increases intracortical facilitation [62], whilst exercise with higher workload, that our V5 condition might be like, could activate wider neural networks and shape neuroplasticity.

In a rehabilitation perspective it has been reported that holding onto the handrails while walking or running on the treadmill reduces the benefits of a workout [9–12]: the use of handrails forces an unnatural walking. On the other hand, patients feel more confident on the treadmill using them, especially if they report medical conditions that interfere with balance. Previous studies suggest that support can improve the safety and stability limit of the center of mass [63, 64]. However, they cannot assess whether these methods induce adaptive changes taking place in the central nervous system.

Nevertheless, an important goal of neurological rehabilitation from a neural perspective is to design exercises stimulating cortical activity and connectivity in regions typically involved in healthy gait, but that can be affected or associated with the site of lesions [65, 66]. The goal of rehabilitation is in fact to provide active exercises capable of inducing improvement in the capacity and quality of movement, and at the same time to induce brain functional reorganization. This study shows that cortical activity in healthy individuals is modulated by varying walking speed, especially when not holding onto the treadmill handrails. Additionally, stronger connectivity is associated with increased speed and the absence of support. Our results indicate that walking speed, and whether holding the treadmill handrail should be considered when planning a treatment to increase cortical activity in regions of interest.

Based on the present results, we propose that, when possible, the handrails should be used only when getting onto and off of the treadmill and when it is starting the movement, but not during walking. At the same time, walking speed should be set close to the maximum gait capacity.

A limitation of this study is the absence of behavioral data to correlate with the hemodynamic responses or to assess the workload associated with the different walking speeds. However, it is worth noting that walking at 3 and 5 km/h is not a demanding task for healthy subjects, therefore behavioral differences between these conditions were not of interest in this study. Nonetheless, this could be relevant for patients with lower limb disability, for whom a speed of 5 km/h could already represent a challenging task, potentially leading to observable differences in behavioral and metabolic aspects.

Further studies need to investigate the cortical correlates of speed and the use of handrails during treadmill walking in a population of patients with neurological diseases affecting gait and balance.

## Conclusions

We demonstrated that walking speed and the use of treadmill handrails influence walking cortical activity patterns, therefore they are key ingredients to take into account when planning a rehabilitation program. It would be advisable not to use the handrails, or gradually reduce their use, in order to keep the body upright and not leaning. Also, the speed should be set as high as possible, gradually increasing it on the basis of individual's performance. We suggest that the effects of walking speed and handrails use on cortical activation levels can significantly contribute to the induction of neural plasticity in the context of repeated intervention sessions. This phenomenon is essential for optimizing therapeutic effects, promoting neurophysiological adaptations that can improve motor function and facilitate recovery. Therefore, modulation of these variables during rehabilitation sessions may represent a strategic approach to maximize clinical outcomes.

### Abbreviations

| | |
|---|---|
| BA | Brodmann's Area |
| FC | Functional connectivity |
| fNIRS | functional near–infrared spectroscopy |
| HbO | Oxyhemoglobin |
| HbR | Deoxyhemoglobin |
| HRF | Hemodynamic response function |
| SS | Short–separation |

### Author contributions

MoBi: Methodology; Investigation; Writing - Original Draft; Visualization. CI: Methodology; Software; Writing - Review & Editing; Visualization. DC: Conceptualization; Writing - Original Draft; Funding acquisition. SC: Methodology; Formal analysis; Writing - Review & Editing. AB: Formal analysis; Writing - Review & Editing. LP: Investigation; Writing - Review & Editing; Visualization. AT: Conceptualization; Writing - Review & Editing. MB: Conceptualization; Methodology; Formal analysis; Writing - Original Draft; Supervision. LB: Conceptualization; Methodology; Formal analysis; Investigation; Writing - Original Draft; Supervision; Funding acquisition.

### Funding

This work was supported by Italian Multiple Sclerosis Foundation (FISM) Grant 2022/R-Multi/021.

### Data availability

The data supporting the findings of this study are not publicly available at present due to the terms of the ongoing grant. However, data may be made available upon reasonable request to the corresponding author at the conclusion of the grant period, after evaluation of possible need to submit a formal project outline or the requirements for co-authorship.

## Declarations

### Ethics approval and consent to participate

The study was approved by the University Ethics Committee (CERA), Genoa, Italy (N. 2023/44). The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All participants provided informed consent to participate in the study.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

Received: 18 September 2024 / Accepted: 3 January 2025
Published online: 16 January 2025

### References

1. Montero-Odasso M, Verghese J, Beauchet O, Hausdorff JM. Gait and cognition: a complementary approach to understanding brain function and the risk of falling. J Am Geriatr Soc. 2012;60(11):2127–36.
2. Nonnekes J, Goselink RJM, Růzicka E, Fasano A, Nutt JG, Bloem BR. Neurological disorders of gait, balance and posture: a sign-based approach. Nat Rev Neurol. 2018;14(3):183–9.
3. Stevens JA, Corso PS, Finkelstein EA, Miller TR. The costs of fatal and non-fatal falls among older adults. Inj Prev. 2006;12(5):290–5.
4. Macko RF, Ivey FM, Forrester LW. Task-oriented aerobic exercise in chronic hemiparetic stroke: training protocols and treatment effects. Top Stroke Rehabil. 2005;12(1):45–57.
5. Teixeira da Cunha Filho I, Lim PAC, Qureshy H, Henson H, Monga T, Protas EJ. A comparison of regular rehabilitation and regular rehabilitation with supported treadmill ambulation training for acute stroke patients. J Rehabil Res Dev. 2001;38(2):245–55.
6. Buurke TJW, Lamoth CJC, Van Der Woude LHV, Den Otter R. Handrail holding during treadmill walking reduces locomotor learning in able-bodied persons. IEEE Trans Neural Syst Rehabil Eng. 2019;27(9):1753–9.
7. Jeka JJ, Lackner JR. Fingertip contact influences human postural control. Experimental Brain Res Exp Brain Res. 1994;100(April):495–502.
8. Russo M, Lee J, Hogan N, Sternad D. Mechanical effects of canes on standing posture: beyond perceptual information. J Neuroeng Rehabil [Internet]. 2022;19(1):1–13. Available from: https://doi.org/10.1186/s12984-022-01067-7
9. Owings TM, Grabiner MD. Variability of step kinematics in young and older adults. Gait Posture. 2004;20(1):26–9.
10. Stephenson JL, Lamontagne A, De Serres SJ. The coordination of upper and lower limb movements during gait in healthy and stroke individuals. Gait Posture. 2009;29(1):11–6.
11. Chang MD, Shaikh S, Chau T. Effect of treadmill walking on the stride interval dynamics of human gait. Gait Posture. 2009;30(4):431–5.
12. Ijmker T, Lamoth CJ, Houdijk H, Tolsma M, Van Der Woude LHV, Daffertshofer A et al. Effects of handrail hold and light touch on energetics, step parameters, and neuromuscular activity during walking after stroke. J Neuroeng Rehabil [Internet]. 2015;12(1):1–12. Available from: https://doi.org/10.1186/s12984-015-0051-3

13. Kang KW, Lee NK, Son SM, Kwon JW, Kim K. Effect of handrail use while performing treadmill walking on the gait of stroke patients. J Phys Ther Sci. 2015;27(3):833–5.
14. Bello O, Marquez G, Camblor M, Fernandez-Del-Olmo M. Mechanisms involved in treadmill walking improvements in Parkinson's disease. Gait Posture [Internet]. 2010;32(1):118–23. Available from: https://doi.org/10.1016/j.gaitpost.2010.04.015
15. Kim HY, Kim EJ, You JH. Adaptive locomotor network activation during randomized walking speeds using functional near-infrared spectroscopy. Technol Heal Care. 2017;25(S1):S93–8.
16. Bakker M, De Lange FP, Helmich RC, Scheeringa R, Bloem BR, Toni I. Cerebral correlates of motor imagery of normal and precision gait. NeuroImage. 2008;41:998–1010.
17. Hamacher D, Herold F, Wiegel P, Hamacher D, Schega L. Brain activity during walking: A systematic review. Neurosci Biobehav Rev [Internet]. 2015;57:310–27. Available from: https://doi.org/10.1016/j.neubiorev.2015.08.002
18. la Fougère C, Zwergal A, Rominger A, Förster S, Fesl G, Dieterich M et al. Real versus imagined locomotion: A [18F]-FDG PET-fMRI comparison. Neuroimage [Internet]. 2010;50(4):1589–98. Available from: https://doi.org/10.1016/j.neuroimage.2009.12.060
19. Vitorio R, Stuart S, Rochester L, Alcock L, Pantall A. fNIRS response during walking — Artefact or cortical activity? A systematic review. Neurosci Biobehav Rev [Internet]. 2017;83(October):160–72. Available from: https://doi.org/10.1016/j.neubiorev.2017.10.002
20. Miyai I, Tanabe HC, Sase I, Eda H, Oda I, Konishi I, et al. Cortical mapping of gait in humans: a near-infrared spectroscopic topography study. NeuroImage. 2001;14(5):1186–92.
21. Oh S, Song M, Kim J. Validating attentive locomotion training using interactive treadmill: an fNIRS study. J Neuroeng Rehabil. 2018;15(1):1–11.
22. Perrey S. Possibilities for examining the neural control of gait in humans with fNIRS. Front Physiol. 2014;5(MAYMay):10–3.
23. Clark DJ, Christou EA, Ring SA, Williamson JB, Doty L. Enhanced somatosensory feedback reduces prefrontal cortical activity during walking in older adults. Journals Gerontol - Ser Biol Sci Med Sci. 2014;69(11):1422–8.
24. Suzuki M, Miyai I, Ono T, Oda I, Konishi I, Kochiyama T, et al. Prefrontal and premotor cortices are involved in adapting walking and running speed on the treadmill: an optical imaging study. NeuroImage. 2004;23(3):1020–6.
25. Schimpl M, Moore C, Lederer C, Neuhaus A, Sambrook J, Danesh J et al. Association between walking speed and age in healthy, free-living individuals using mobile accelerometry-a cross-sectional study. PLoS ONE. 2011;6(8).
26. Lo OY, Halko MA, Zhou J, Harrison R, Lipsitz LA, Manor B. Gait speed and gait variability are associated with different functional brain networks. Front Aging Neurosci. 2017;9(NOV).
27. Kim J, Lee J, Lee G, Chang WH, Ko MH, Yoo WK, et al. Relationship between lower limb muscle activity and cortical activation among elderly people during walking: effects of fast speed and cognitive dual task. Front Aging Neurosci. 2023;14(January):1–14.
28. Mathôt S, Schreij D, Theeuwes J, OpenSesame. An open-source, graphical experiment builder for the social sciences. 44, Behav Res Methods. 2012.
29. Peirce JW. PsychoPy-Psychophysics software in Python. J Neurosci Methods. 2007;162:1–2.
30. Kothe C. Lab streaming layer (LSL). https://githubcom/sccn/labstreaminglayer Accessed Oct. 2014;26.
31. Brigadoi S, Cooper RJ. How short is short? Optimum source–detector distance for short-separation channels in functional near-infrared spectroscopy. Neurophotonics. 2015;2(2):025005.
32. Huppert TJ, Diamond SG, Franceschini MA, Boas DA. HomER: a review of time-series analysis methods for near-infrared spectroscopy of the brain. Appl Opt. 2009;48(10).
33. Selb J, Yücel MA, Phillip D, Schytz HW, Iversen HK, Vangel M, et al. Effect of motion artifacts and their correction on near-infrared spectroscopy oscillation data: a study in healthy subjects and stroke patients. J Biomed Opt. 2015;20(5):056011.
34. Cooper RJ, Selb J, Gagnon L, Phillip D, Schytz HW, Iversen HK, et al. A systematic comparison of motion artifact correction techniques for functional near-infrared spectroscopy. Front Neurosci. 2012;6(OCT):1–10.
35. Iester C, Bonzano L, Biggio M, Cutini S, Bove M, Brigadoi S. Comparing different motion correction approaches for resting-state functional connectivity analysis with functional near-infrared spectroscopy data. Neurophotonics. 2024 Oct;11(4):045001. https://doi.org/10.1117/1.NPh.11.4.045001. Epub 2024 Oct 3. PMID: 39372120; PMCID: PMC11448702.
36. Di Lorenzo R, Pirazzoli L, Blasi A, Bulgarelli C, Hakuno Y, Minagawa Y, et al. Recommendations for motion correction of infant fNIRS data applicable to multiple data sets and acquisition systems. NeuroImage. 2019;200:511–27.
37. Brigadoi S, Ceccherini L, Cutini S, Scarpa F, Scatturin P, Selb J, et al. Motion artifacts in functional near-infrared spectroscopy: a comparison of motion correction techniques applied to real cognitive data. NeuroImage. 2014;85:181–91.
38. Yücel MA, Selb J, Cooper RJ, Boas DA, TARGETED PRINCIPLE COMPONENT ANALYSIS:. A NEW MOTION ARTIFACT CORRECTION APPROACH FOR NEAR-INFRARED SPECTROSCOPY. J Innov Opt Health Sci. 2014;7(2).
39. Scholkmann F, Spichtig S, Muehlemann T, Wolf M. How to detect and reduce movement artifacts in near-infrared imaging using moving standard deviation and spline interpolation. Physiol Meas. 2010;31(5):649–62.
40. Molavi B, Dumont GA. Wavelet-based motion artifact removal for functional near-infrared spectroscopy. Physiol Meas [Internet]. 2012;33(2):259–70. Available from: https://iopscience.iop.org/article/https://doi.org/10.1088/0967-3334/33/2/259
41. Scholkmann F, Wolf M. General equation for the differential pathlength factor of the frontal human head depending on wavelength and age. J Biomed Opt [Internet]. 2013;18(10):105004. Available from: https://doi.org/10.1117/1.JBO.18.10.105004
42. Delpy DT, Cope M, Zee P, van der, Arridge S, Wray S, Wyatt J. Estimation of optical pathlength through tissue from direct time of flight measurement. Phys Med Biol [Internet]. 1988;33(12):1433–42. Available from: https://doi.org/10.1088/0031-9155/33/12/008
43. Barker JW, Aarabi A, Huppert TJ. Autoregressive model based algorithm for correcting motion and serially correlated errors in fNIRS. Biomed Opt Express [Internet]. 2013;4(8):1366–79. Available from: https://opg.optica.org/boe/abstract.cfm?URI=boe-4-8-1366
44. Bonzano L, Biggio M, Brigadoi S, Pedullà L, Pagliai M, Iester C et al. Don't plan, just do it: cognitive and sensorimotor contributions to manual dexterity. NeuroImage. 2023;280(August).
45. Di Rosa E, Brigadoi S, Cutini S, Tarantino V, Dell'Acqua R, Mapelli D, Braver TS, Vallesi A. Reward motivation and neurostimulation interact to improve working memory performance in healthy older adults: A simultaneous tDCS-fNIRS study. Neuroimage. 2019 Nov 15;202:116062. https://doi.org/10.1016/j.neuroimage.2019.116062. Epub 2019 Jul 29. PMID: 31369810; PMCID: PMC7467146.
46. Diamond SG, Huppert TJ, Kolehmainen V, Franceschini MA, Kaipio JP, Arridge SR, et al. Dynamic physiological modeling for functional diffuse optical tomography. NeuroImage. 2006;30(1):88–101.
47. Zimeo Morais GA, Balardin JB, Sato JR. FNIRS Optodes' Location Decider (fOLD): a toolbox for probe arrangement guided by brain regions-of-interest. Sci Rep. 2018;8(1):1–11.
48. Iester C, Biggio M, Cutini S, Brigadoi S, Papaxanthis C, Brichetto G et al. Time-of-day influences resting-state functional cortical connectivity. Front Neurosci. 2023;17(May).
49. Fisher RA. On the probable error of a coefficient of correlation deduced from a small sample. Volume 9. Biometrika. Metron; 1921. pp. 3–32.
50. Kleinbaum DG, Kupper LL, Muller KE. Applied regression analysis and other multivariable methods. 2nd ed. Duxbury series in statistics and decision sciences TA - TT -. Boston SE - XVIII, 718 p.; 25 cm.: PWS-Kent; 1988.
51. De Jong BM, Leenders KL, Paans AMJ. Right parieto-premotor activation related to limb-independent antiphase movement. Cereb Cortex. 2002;12(11).
52. Dobkin BH, Firestine A, West M, Saremi K, Woods R. Ankle dorsiflexion as an fMRI paradigm to assay motor control for walking during rehabilitation. NeuroImage. 2004;23(1).
53. Mihara M, Miyai I, Hatakenaka M, Kubota K, Sakoda S. Sustained prefrontal activation during ataxic gait: a compensatory mechanism for ataxic stroke? NeuroImage. 2007;37(4).
54. Mihara M, Miyai I, Hatakenaka M, Kubota K, Sakoda S. Role of the prefrontal cortex in human balance control. NeuroImage. 2008;43(2).
55. Harada T, Miyai I, Suzuki M, Kubota K. Gait capacity affects cortical activation patterns related to speed control in the elderly. Exp Brain Res. 2009;193(3).
56. Ptak R. The frontoparietal attention network of the human brain: Action, saliency, and a priority map of the environment. Vol. 18, Neuroscientist. 2012.
57. Kurz MJ, Wilson TW, Arpin DJ. Stride-time variability and sensorimotor cortical activation during walking. NeuroImage. 2012;59(2).
58. Maidan I, Nieuwhof F, Bernad-Elazari H, Reelick MF, Bloem BR, Giladi N et al. The role of the Frontal lobe in Complex walking among patients with Parkinson's Disease and healthy older adults: an fNIRS Study. Neurorehabil Neural Repair. 2016;30(10).

59. Bootsma JM, Caljouw SR, Veldman MP, Maurits NM, Rothwell JC, Hortobágyi T. Failure to Engage Neural Plasticity through Practice of a High-difficulty Task is Accompanied by Reduced Motor Skill Retention in Older Adults. Neuroscience [Internet]. 2020;451:22–35. Available from: https://www.sciencedirect.com/science/article/pii/S0306452220306643
60. Carey JR, Bhatt E, Nagpal A. Neuroplasticity Promoted by Task Complexity. Exerc Sport Sci Rev [Internet]. 2005;33(1). Available from: https://journals.lww.com/acsm-essr/fulltext/2005/01000/neuroplasticity_promoted_by_task_complexity.5.aspx
61. Guadagnoli MA, Lee TD, Challenge Point. A Framework for Conceptualizing the Effects of Various Practice Conditions in Motor Learning. J Mot Behav [Internet]. 2004;36(2):212–24. Available from: https://doi.org/10.3200/JMBR.36.2.212-224
62. Clos P, Lepers R, Garnier YM. Locomotor activities as a way of inducing neuroplasticity: insights from conventional approaches and perspectives on eccentric exercises. 121, Eur J Appl Physiol. 2021.
63. Karpatkin HI, Benson A, Gardner N, Leb N, Ramos N, Xu H et al. Pilot trial of speed-intensive gait training on balance and walking in people with multiple sclerosis. Int J Ther Rehabil. 2020;27(11).
64. Odanye O, Steffensen E, Hinton E, Bierner S, Hsiao HY, Knarr B. Treadmill Handrail-Use increases the Anteroposterior Margin of Stability in individuals' Post-stroke. J Mot Behav. 2024;56(3).
65. Kleim JA, Jones TA. Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. J Speech Lang Hear Res. 2008;51(1):S225–39.
66. Levin MF, Kleim JA, Wolf SL. What do motor recovery and compensation mean in patients following stroke? Neurorehabil Neural Repair. 2009;23(4):313–9.

## Publisher's note