Title: Short-Term Therapeutic Effects of Anti-Gravity Treadmill Training on Brain Functional Activities and Walking Capacity in Children With Cerebral Palsy

Running Title: AlterG Training in Children with CP

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Abstract

Introduction: Cerebral Palsy (CP) is one of the most common causes of motor disability in childhood. Since CP is a corollary to brain damage, persistent treatment should accompany an alteration in brain functional activity in line with clinical improvements.

Methods: Fourteen children with spastic hemiplegia CP were randomly divided into 2 groups. The study group (8yrs-5mos) underwent 45min AlterG training sessions 3 times/week for 8 weeks, while the control group (8yrs-2mos) received the same amount of occupational therapy (OT). Functional magnetic resonance imaging (fMRI) was conducted to quantify brain activation during the performance of passive tasks including ankle plantarflexion to dorsiflexion and knee flexion to extension over the range of motion. Walking capacity was assessed using the Timed-Up-and-Go, 10-meter, and 6-minute walk tests. All evaluations were performed before and after training and compared between the two groups.

Results: We were able to detect the signatures of ankle and knee passive movement tasks in the fMRI and characterize them in terms of activated voxels. The pre-post activation changes following the completion of training course showed that the elicited motor cortex activation was greater for the ankle than the knee tasks. For the ankle, primary motor cortex, precentral gyrus and corpus callosum showed significant enhancement in most study participants. The results indicated 16.1% more active voxels in the study than control groups. Similarly, clinical outcome measures improved over twice as much in this group.

Conclusions: AlterG training could be a potentially effective therapeutic intervention for improving gait and balance impairments in children with CP.

Keywords: Cerebral Palsy (CP), functional Magnetic Resonance Imaging (fMRI), Anti-Gravity Treadmill, Gait

Highlights

- AlterG training is potentially effective physical intervention to improve gait and postural stability in children with CP.
- FMRI, performed with passive tasks, could be an effective tool for detecting alterations in brain activity in children with CP.
- Both brain activity and walking capacity demonstrated greater improvement following the completion of AlterG compared to OT training.

1. Introduction

Cerebral Palsy (CP), one of the most common causes of motor disability in children, is attributed to non-progressive disturbances occurring in the developing fetal or infant brain. Overall, the CP rate is between 2 and 3 per 1000 live births [1] which increases to 40–100 in premature births or infants with very low birth weights [2]. CP belongs to the group of movement disorders which not only limits functional activities and reduces the quality of life, but also imposes economic burden on societies. Hence, an effective intervention is needed to permanently improve their movement and balance, and as a result, to decrease their dependency.

Physical and occupational therapy (OT) usually start in the first few years of life or soon after diagnosis. Despite their popularity, their outcomes are typically limited and short-lasting, indicating inadequate production of neuroplasticity [3,4]. Therapeutic approaches such as muscle injections, drugs, and surgical procedures can reduce spasticity, but mostly have short-term effects and considerable side effects and risk factors [5–8].

An adequate long-term, intensive systematic training is required for efficient neuroplasticity induction [9], resulting in persistent improvement of balance and movement. In this regard, the robotic-assisted locomotor training (LOKOMAT) was developed and used to provide an intensive and systematic training. However, LOKOMAT training was shown to have limited and short-term therapeutic effects since it mostly provides passive training [10]. Alternatively, active body weight-supported treadmill training (BWSTT) has been recently introduced for the purpose of improving gait in individuals with CP. Some studies have shown that BWSTT was effective in enhancing gait speed [11–13], whereas others have reported no superiority of BWST vs conventional gait training [14,15]. Although

improvements were widely reported in gait endurance and speed [16], groin discomfort and skin irritation could be the restriction factors of BWSTT.

A recently developed anti-gravity treadmill (AlterG) [17] can address the issues associated with BWSTT and facilitate treadmill walking because of its capacity to substantially reduce lower extremity weight bearing [1,18]. Using precise unweighting technology, the anti-gravity treadmill can help individuals walk actively on the treadmill with adjustable speed [1]. This can trigger neural messaging in motor and sensory pathways, which may result in brain reorganization. If brain neuroplasticity is induced and correlated with clinical improvements, long-lasting effects would be expected.

The characterization of therapy-induced neuroplasticity in addition to functional improvement is of invaluable importance. This not only contributes to understanding the efficacy of the therapy, and underlying mechanisms of the disease, but also ensures that clinically significant enhancement of functional outcomes are persistent. Considering this, neuroimaging modalities including structural and diffusion magnetic resonance imaging (MRI) and functional MRI (fMRI) are vital for identifying neuroplasticity [1,19–24]. FMRI detects changes in blood flow (hemodynamic response) associated with neural activation of the brain [25,26]. FMRI uses blood-oxygen-level-dependent (BOLD) contrast and can measure BOLD responses of task-induced activities. One very popular modality used to measure neuroplasticity is task-based functional MRI (t-fMRI) which is widely adopted for the identification of brain regions that are functionally involved in performing a specific task during data acquisition [22,27–29].

Few studies to date have used task-based fMRI to investigate therapy-driven neuroplasticity in gait rehabilitation in children with spastic hemiplegic CP due to technical challenges [30,31]. However, several small-scale studies have reported therapy-induced fMRI-detected changes in children with unilateral CP (UCP) [31,32]. Since fMRI acquisition requires subjects to remain fixed in a restricted space for a long period of time, motion artifacts in fMRI scans limited the feasibility of standard analysis in these studies and even studies on healthy pediatrics [31–33].

To overcome concerns regarding motion artifacts, subjects generally need to be sedated with an ideal sedative agent, which minimally hampers the neurophysiologic effect of the administered sensory and motor stimulation [34,35]. There are successful reports on the fMRI of sedated patients performed with passive tasks including motion of the extremities

[36–38]. Passive movements were able to activate most of the cortical areas traditionally described in motor systems [39,40], such as the contralateral sensorimotor cortex, particularly the premotor cortex and parietal cortex [18]. Accordingly, we aimed to (1) detect the signatures of ankle and knee passive movement tasks in the fMRI of children with CP, (2) characterize these signatures, and (3) investigate the effects of AlterG training on these signatures, as well as on walking capacity in children with CP. We hypothesized that intensive and systematic AlterG training, which induces active walking, may result in enhancement of brain functional activities and long-term improvement of gait and balance impairments.

2. Materials and Methods

2.1 Participants

Fourteen subjects with spastic hemiplegia CP (6 – 11 years old) were included in this study; they were randomly divided into 2 groups. The study group (5 females and 2 males; mean age [SD] 8y 5mo [1y 11mo], range 6y-11y) underwent anti-gravity treadmill training and the control group (5 females and 2 males; mean age [SD] 8y 2mo [1y 4mo], range 7y-10y) received OT. There was no significant difference in sex and age between the study and control groups. The inclusion criteria: hemiplegic, having spasticity in the lower limb (modified ashworth>1), ability to stand independently for at least 30 seconds, and ambulatory. The exclusion criteria: severe cognitive deficits, received botulinum toxin injections within the past 2 months, and a history of surgery 6 months prior to training. All parents/guardians provided a written informed consent, and the study had ethical approval from the Tehran University of Medical Sciences (TUMS) ethic committee.

2.2 Training Protocol

2.2.1 AlterG anti-gravity treadmill

The AlterG anti-gravity treadmill (F320, AlterG® California USA) uses an inflatable tent surrounding the lower extremities to exert an upward force on the lower body and thereby reducing ground reaction forces in the lower limbs (Figure 1). This allowed the body weight to be reduced up to 80% by 1% increments.

The anti-gravity treadmill training was performed for 45 minutes per session, 3 days/week for 8 weeks. In each session, the training started with a speed of 1km/h and a body weight support of 50%. Then, the body weight support gradually decreased and the speed increased

as needed based on the subject's ability and the discretion of the physical therapist who was responsible for the training. The subject was provided with necessary feedback by the trainer [1,18,40].

2.2.2 Occupational Therapy Training

A pediatric occupational therapist provided the control group with OT, with a focus on balance and gait training, at the rehabilitation center. OT training mostly concentrated on locomotion and was performed in 45 min sessions, 3 days per week for 8 weeks, similar to the training program of the study group.

2.3 Image Acquisition

Participants underwent MRI scans before and after the 8-week training programs. All children were sedated under the supervision of pediatric anesthesiologists prior to undergoing MRI. The default sedation protocol involved general anesthesia with intra-venous propofol administered at the lowest dose to keep the patient asleep after induction. For all subjects, oxygen saturation and heart rate were monitored by pulse-oximetry throughout the examination. Information regarding anesthesia, including the medications used for induction and maintenance, was recorded in the medical record.

2.3.1 Tasks Description

Task-based fMRI (t-fMRI) is a non-invasive technique, which allows for identification of brain regions with altered activity due to the performance of given tasks. T-fMRI experiments obtained from high-resolution scans provide hundreds of thousands of longitudinal signals for each individual, corresponding to measurements of brain activity for each voxel of the brain over the duration of the experiment.

In this study, fMRI acquisition included two passive motor tasks for each leg: (1) ankle plantarflexion to dorsiflexion movements over the range of motion (ROM) with 1Hz frequency (Figure 2a), and (2) knee flexion to extension movements over the ROM with 0.5Hz frequency (Figure 2b). All passive tasks were performed by a trained biomedical engineer. A block design was used for fMRI studies with 24-second periods of rest alternating with 24-second periods of motor task for a total of 5 cycles.

2.3.2 Scanning Parameters

FMRI data were acquired using a 3-Tesla GE MRI scanner with a standard head coil and single-shot gradient echo-planar imaging (matrix=64×64, TE=30ms, TR=3000ms) to obtain

80 images with a 3mm slice thickness. High resolution anatomic T1-weighted images were obtained (matrix=192×192, TE=3.44ms, TR=1800ms) with a slice thickness of 1mm. Structural and functional images were obtained in an axial direction parallel to the anterior/posterior commissure line.

2.4 FMRI Processing

The preprocessing and statistical analysis of the fMRI data were calculated with functional magnetic resonance imaging of the brain (FMRIB) software library (FSL v6.02). FMRI data was preprocessed using the standard steps including realignment, brain extraction, motion correction, spatial smoothing, filtering and denoising. Since transformation of the functional data into standard space can affect functional analysis outcomes and group difference determinations, the standard space (MNI152 atlas) is registered to functional data [41]. The region of interest (ROI) analysis used in this study included the primary motor cortex (M1), premotor cortex (PMC), supplementary motor area (SMA), precentral gyrus (PG) and corpus callosum (CC), which was selected from the Harvard-Oxford probabilistic atlas, and subsequently transformed to the individual's native space.

The images were realigned and coregistered to the mean functional image from the first session. We followed the FSL procedure to produce a non-brain mask for brain extraction. The movement parameters of the subjects were included in the individual analysis as covariates of no interest in order to reduce motion artifacts. FMRI images were then smoothed with a Gaussian kernel by a 5×5×5mm full width half maximum (FWHM) and a high pass filter of 72s. The primary goal of spatial smoothing is to enhance the signal to noise ratio (SNR) and suppress spatial noise. Denoising was performed using multivariate exploratory linear optimized decomposition into independent components (MELODIC). Noisy components with voxels outside the brain were considered artifacts and removed from the data. Independent component analysis (ICA) was rerun for each subject's temporally concatenated data across all sessions [42–44].

First-level individual statistical analyses were performed to calculate the significant brain areas, using the general linear model (GLM). Second-level random effects models were used to estimate brain activation for the separate contrasts (passive movements versus rest) and for each group. Then t-test is used to detect significant differences in study group,

compared to control group for passive movements > rest, contrast. All contrasts were reported for clusters comprising at least 10 voxels and false discovery rate (FDR), $p < 0.05$.

2.5 Clinical Evaluation of Gait and Balance

The common clinical measures used to evaluate walking capacity included:

1) Ten-meter walk test (10MWT) to assess walking speed [45], by measuring the duration of a 10-m walk.
2) Six-minute walk test (6MWT) to evaluate walking endurance [46], by measuring the distance walked in 6min.
3) Timed-Up-and-Go (TUG) to assess balance and mobility [47], by measuring the duration of the required task, including standing, walking, and sitting back.

3. Results

3.1 Therapeutic Effects on Functional Brain Activity

3.1.1 Individual Results

Our results revealed that both ankle and knee passive motor tasks can activate the motor cortex in both the study and control groups. We were able to successfully detect the signatures of these tasks in the fMRI of CP children. We characterized these signatures in terms of activated voxels. Table 1 summarizes the pre- and post-results of the motor cortex activation analysis in terms of the number of active voxels for all tasks in both the study and control groups. The therapeutic effects of the AlterG training on the activated voxels present several major points in the study group:

1. The passive tasks of the ankle resulted in greater motor cortex activation compared to those of the knee.
2. The pre-post activation changes in the motor cortex, were observed for the passive ankle tasks of both sides in all but participant 4, in which the activation was elicited by the affected ankle after treatment.
3. In participants 1 and 2, the activation simultaneously increased in both the left and right hemispheres, in response to the passive movement of each ankle.
4. In participants 3 and 5, the activation increased in the less affected side but decreased in the more affected side.

5. In participant 6, passive movement of the more affected ankle resulted in an increase in the activation of the contralateral hemisphere and a decrease in the ipsilateral hemisphere. Whereas, the passive movement of the less affected ankle led to reduced activation of both hemispheres.

Participant 7 was excluded due to the patient's refusal to attend the 2nd fMRI session.

In the control group, the pre-post activation improvement from OT training, was seen mostly in participant 11. In contrast, the pre-post activation substantially reduced following the training. Participant 14 was excluded because of excessive head movement during fMRI acquisition.

To further analyze the pre-post activation changes in the motor cortex, we divided the motor cortex into distinct ROIs. Table 2 describes the distinct ROIs with significant activation alterations in response to therapy along with the local maxima in MNI coordinates as well as the most activated clusters for each participant. The ROI-based analysis revealed more interesting results; overall, PMC, PG and CC showed significant enhancement in terms of activated voxels in most participants while significant improvement in SMA and M1 were only observed in half of the subjects.

The details of the ROI analysis revealed several major points in the study group:

1. A significant increase in the activation of PMC and CC regardless of the affected sides.
2. In participants with increased activation in the PMC of one side, the more affected hemisphere showed more improvement.
3. In most participants with increased activation in the PMC of both sides, the enhancement was greater in the more affected side as compared to the less affected side.
4. In all participants but case 1, a significant increase was seen in the activation of M1. In participants with increased activation of M1 in both hemispheres, the more affected side showed greater improvement.
5. All participants except case 5 revealed a significant increase in the activation of PG.
6. In participants 2, 3 and 5, a significant activation of SMA was observed; interestingly, there was concurrent improvement in the PMC of both hemispheres as well.

In the control group, participants 8, 9 and 13 (half of the control group) showed little improvement only in a limited number of regions, for which the study group demonstrated considerable improvement. Furthermore, in contrast to the study group, the activation enhancement was detected in the PMC of both sides of the remaining half, accompanied by similar changes in PG and SMA.

3.1.2 Group Results

Figure 3 shows the average group results of significant activation changes following the completion of the 8-week training course for the ROI regions of both the study and control groups as well as the group differences. The results revealed significant differences in brain activation for both groups (Fig. 3a and 3b). However, the changes were observed in both hemispheres in the study group, whereas it was observed mostly in one of the hemispheres of the control group. Furthermore, the areas with significant changes were substantially larger in the study group than in the control group.

For a more precise description of these findings, the differences in the significant activation changes between the study and control groups were obtained. The results showed 16.1% more active voxels in the study group compared to that of the control group (Fig. 3c).

3.2 Therapeutic Effects on Balance and Gait Impairment

Table 3 summarizes the average group results of the clinical measures of walking capacity and the percentage of changes after the administration of the 8-week training course for both the study and control groups. Walking speed increased by 36.3% and 24.9% for the study and control groups, respectively. Importantly, balance and mobility showed a much larger improvement in the study group compared to the control group; 37.2% vs. 16.8%. More importantly, walking endurance enhanced three times more in the study group than in the control group.

4. Discussion

For the first time, this article characterizes the therapeutic effects of intensive AlterG training on brain cortical reorganization and walking capacity in children with CP using passive task-based fMRI under sedation. Our aim was to study the responsiveness of motor cortical reorganization of children with hemiplegic CP after anti-gravity treadmill training versus over-ground walking OT. Passive movements included dorsiflexion and plantar

flexion of both ankles and flexion-extension of both knees, which were carried out on all subjects before and after the 8-week training; this enabled us to investigate the intra-subject brain activation alterations induced by therapy as well as the group comparison. Our findings indicated a higher enhancement in both brain activity and walking capacity following the completion of AlterG in comparison to OT training, suggesting that AlterG training may be used as an effective therapeutic intervention for long-lasting improvement of gait and balance impairments in children with CP.

High sensitivity of task-based fMRI to motion artifacts could worsen in children with CP who neither cooperate to lie down immobile nor perform tasks correctly during fMRI acquisition. A similar study highlighted the difficulties of fMRI scanning in children with CP [48]. To address this issue, in this study fMRI was acquired under sedation during which the passive tasks were applied. To the best of authors' knowledge this was done for the first time; we found no evidence of sedative passive task-based fMRI in children with CP, although some studies investigated passive tasked-based fMRI in this patient population [49].

To consider the heterogeneity of the size and location of the brain lesions in CP participants included in this study, we used the ROI analyses to measure the therapy-induced fMRI-detected changes of brain activation between the motor cortices before and after training in each subject. The percentage of changes in the outcome measures were calculated for each subject in each study and control group. Then, the group average results were used to determine the impact of each intervention and compare the effectiveness of the interventions.

The activation patterns for different tasks were compared using the same data acquisition parameters, and analysis methods for both the intra- and inter-subject and group analyses on the FDR ($p<0.05$) corrected level were employed. Subsequent data analysis and comparisons are the first to suggest that motor cortical activation increases after 8 weeks of anti-gravity treadmill training and OT training. Moreover, the changes in therapy-driven motor cortical activation were more widely distributed with higher intensity in the study group compared to the control group. Passive movement tasks used in this study produced consistent activation in the motor system of both legs and demonstrated robust activation in M1, PMC, SMA and PG of both hemispheres as well as the CC which connects the left and right cerebral hemispheres. This distribution of brain activity following sedative passive task-

based fMRI is in accordance with previous studies on sedative-free passive task-based fMRI movements in healthy adults [38], adult stroke patients [50], healthy children [51] and children with CP [49]. This might imply that the supraspinal sensorimotor network for the neural control of walking can be assessed indirectly by these tasks.

According to Table 1, in 3 subjects of the study group (i.e. subjects 3, 5, and 6), the activation of motor areas induced by training increased in one hemisphere but decreased in the other. This was observed in only one task in one control patient (subject 9, left knee task). In subject 3, brain activation due to 3 tasks increased in the left hemisphere and CC, but decreased in right hemisphere. In subject 5, for 3 tasks, brain activation increased in the contralateral side and decreased in the ipsilateral side and the CC. In subject 6, in the left knee task, brain activation increased in the contralateral side and decreased in the ipsilateral side and the CC. These contradictory activation changes in the hemispheres suggest that the investigated motor areas in one hemisphere may adaptively compensate for the other.

Studies that utilized anti-gravity treadmill training, BWST, and LOKOMAT for gait improvement, mostly reported functional improvement rather than characterization of therapy-induced brain reorganization in children with CP [1,13,51,52]. However, few studies have investigated the therapy-driven neuroplasticity in gait rehabilitation using BWST in adults with stroke and children with CP [29,49–50]. The hemodynamic response of the sensorimotor cortices following therapy has been reported to increase in some of these fMRI studies, while others showed that brain cortical activation decreased [49,51]. Furthermore, according to a limited number of small-scale fMRI investigations in children with UCP, increased contralateral activity may accompany functional gains. For instance, cluster-based S1–M1 voxel counts were increased after virtual reality therapy in three adults with UCP [50]. However, our intra-subject analysis demonstrates both an increase and decrease in motor cortical activation after therapy. This might be due to the initial severity of the sensorimotor impairments evident in participants.

Although both groups demonstrated an improvement in walking speed, TUG, and walking endurance, participants in the study group had much greater enhancement. This is concurrent with higher brain motor cortical reorganization induced in the study group. This implies that AlterG training may have the potential to promote effective neuroplasticity that can improve walking ability in children with CP.

5. Limitation

In this study, the therapeutic effects of AlterG training on brain functional activity and walking capacity were successfully characterized. While our results were promising with respect to the investigation of therapy-driven improvement of functional brain activities, our study had a few limitations. Firstly, few patients could not complete the required training sessions due to the intensive treatment schedule.

Secondly, our results showed no significant correlation between these measurements (secondary aim), probably due to the limited sample size, which can mostly influence this aim, but not the primary ones. Our major objectives were firstly to examine the possibility of detecting the signatures of ankle and knee passive movement tasks in the fMRI of CP children, and if so, secondly to characterize these signatures, and finally to determine the potential therapeutic effects of the antigravity treadmill training on these signatures, and of course on balance and gait impairments. We were able to detect these signatures (aim1), particularly for the ankle task, characterize them in terms of activated voxels (aim2), and determine the therapeutic effects of trainings on the activated voxels (aim3). Our findings revealed different therapeutic responses following the completion of trainings, consistent with the literature reporting a high inter-subject variability in brain structural and functional neuroplasticity due to several factors.

Finally, characterization of the intervention effects may not solely be achieved by the pre-post analyses and calculation of the average group results. Alternatively, since any intervention can have different effects on patients, the recovery patterns need to be identified to fully characterize the therapeutic effects of interventions and individualize treatment. This required a larger sample size and further data acquisition time points, considered in our ongoing studies.

6. Conclusion

The findings of this study demonstrate brain activation enhancement following the administration of the 8-week AlterG training in children with CP. This implies that AlterG training can be considered as an effective physical intervention to improve walking capacity in children with CP. Our results also indicate that fMRI, performed with passive tasks, is an effective tool for detecting alterations in brain activity induced by physical activities in children with CP.

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This study is registered in the Iranian Registry of Clinical Trials (IRCT) with number IRCT2015121625568N1. This article has been extracted from the thesis written by Mr. Meghdad Ashtiyani in Biomedical Engineering and Medical Physics Department, School of Medicine, Shahid Beheshti University of Medical Sciences (Registration No: 365m).

8. Authors' contributions

Principal Investigator and study design: M.M.Mirbagheri and M.R.Deevband; Methodology: M.Ashtiyani, M.M.Mirbagheri, A.Shahrokhi and M.R.Deevband; Acquisition of data: M.Ashtiyani and P.M.Birgani; Data analysis: M.Ashtiyani, P.M.Birgani; Interpretation of the findings: M.Ashtiyani, M.M.Mirbagheri, B.Jameie and M.soleimani; Writing and preparing manuscript: M.Ashtiyani, P.M.Birgani; Reviewing and approving the final version for publication: All authors.

Conflict of Interest: The authors expressed no conflicts of interest for the authorship, research, and publication of this study.

Ethics Approval: The study was approved by the ethical committee 'Tehran University of Medical Sciences (TUMS)'. All participants gave their written informed consent to participate in the study.

Data Availability: Data are available upon reasonable request.

Code availability: N/A

Consent to Participate: Written informed consent of the parents of patients was collected.

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Table 1: Number of active voxels in the motor cortex (brain areas related to planning (PMC), control (M1), and execution of movements (SMA and PG) at $z>3.1$ and $p<0.05$. (LF: left foot, RF: right foot, LK: left knee, RK: right knee)

Training	Case	Task / Brain side	RF		LF		RK		LK	
			R	L	R	L	R	L	R	L
Anti-Gravity Treadmill	1	Pre	213	408	192	260	0	0	0	0
		Post	393	652	359	375	0	72	0	0
	2	Pre	74	155	42	292	-	-	-	-
		Post	33	248	54	341	-	-	-	-
	3	Pre	74	67	255	60	357	112	-	0
		Post	46	515	83	196	-	-	0	397
	4	Pre	0	0	0	0	0	0	0	0
		Post	86	0	0	0	87	0	51	0
	5	Pre	190	322	144	199	24	203	86	32
		Post	113	434	272	187	-	-	229	0
	6	Pre	416	513	92	134	0	0	0	0
		Post	95	108	202	79	51	0	0	0
Occupational Therapy	8	Pre	0	61	0	25	33	66	68	66
		Post	22	102	0	0	46	79	0	83
	9	Pre	0	0	0	190	0	0	0	0
		Post	56	0	0	0	0	0	0	0
	10	Pre	0	0	0	0	33	0	0	0
		Post	510	352	309	30	52	0	49	0
	11	Pre	246	223	188	394	171	0	54	39
		Post	1044	1166	194	0	448	495	0	0
	12	Pre	0	70	247	137	0	0	0	0
		Post	199	212	0	0	79	66	121	108
	13	Pre	206	135	488	424	185	480	59	139
		Post	0	55	186	143	0	0	0	0

Table 2: Brain regions with significant difference in activation between pre- and post-treatment ($p<0.05$)

	Subject	z	-10 log (p value)	Max (x , y , z)	# of clusters	Cluster Size	Brain area
Anti-gravity treadmill training	1	12.2	33.2	(-16,-31,60)	1	5225	Precentral gyrus
		5.9	1.32	(-22,-37, 66)	2	99	left premotor cortex
		14.8	27.2	(-30,-34 ,14)	1	3886	Corpus callosum
	2	6.91	36.1	(6, -46, 66)	1	709	Right primary motor cortex
		7.01	46.6	(-22,-44,68)	3	1042	left primary motor cortex
		6.1	39.7	(20,-36,70)	1	2176	Right premotor cortex
		7.58	4.12	(-66,-4,10)	2	104	left premotor cortex
		6	18.6	(-6,-18,74)	1	720	Supplementary motor cortex
		7.78	66.7	(-68,-4,10)	2	4693	Precentral gyrus
		6.48	18.7	(2,-30,28)	4	761	Corpus callosum
	3	6.22	12.5	(52,-14,28)	1	651	Right primary motor cortex
		9.27	20.7	(-52,-4,32)	2	1351	left primary motor cortex
		8.56	19.4	(4,-24,40)	2	1253	Right premotor cortex
		9.27	32.4	(-52,-4,32)	1	2612	left premotor cortex
		7.23	16.8	(-10,-20,40)	1	995	Supplementary motor cortex
		10.3	53.5	(-56,20,10)	2	5012	Precentral gyrus
		9.48	60.7	(-38,-52,-4)	1	6618	Corpus callosum
	4	9.21	16.3	(4, -43, 60)	1	321	Right primary motor cortex
		7.6	19.1	(22,-30,68)	1	826	Right premotor cortex
		4.07	2.2	(62, 14, 8)	1	149	Precentral gyrus
		5.41	7.1	(13,-30,20)	2	432	Corpus callosum
	5	8.25	4.66	(40, -28, 70)	1	603	Right primary motor cortex
		6.14	19.3	(-6, -18, 40)	2	817	left primary motor cortex
		8.25	5.21	(40, -28, 70)	1	1423	Right premotor cortex
		7.88	2.29	(-22, 28, 62)	5	59	left premotor cortex
		7.5	16.2	(12, -6, 72)	2	636	Supplementary motor cortex
		7.4	26.1	(24, -18, 32)	1	1306	Corpus callosum
	6	11	2.15	(62, -10, 42)	1	21	Right primary motor cortex
		5.59	1.3	(66, 12, 24)	1	56	Right premotor cortex
		7.73	3.71	(62, 20, 14)	1	182	Precentral gyrus
		4.27	1.95	(6, -4, 24)	1	93	Corpus callosum
Occupational Therapy	8	4.93	2.79	(13, 0, 36)	2	56	Right premotor cortex
		3.21	7.03	(-3, 7, 44)	1	199	Precentral gyrus
		4.89	1.51	(-10,22,15)	3	412	Corpus callosum
	9	3.3	2.72	(-16,-52,12)	2	170	Corpus callosum
	10	8.92	15.6	(-10,-36,68)	1	1708	Right primary motor cortex
		8.7	19.6	(-26,-38,72)	1	2421	left primary motor cortex
		5.17	2.2	(6, -32, 68)	2	2992	Right premotor cortex
		8.22	22	(-26,-36, 68)	1	2914	left premotor cortex
		7.75	17.3	(6, -20, 76)	1	2004	Supplementary motor cortex
		9.45	42	(68, 2, 10)	1	7569	Precentral gyrus
		8.34	39.5	(-4, -44, 6)	1	6902	Corpus callosum
	11	10.6	11.2	(16, -24, 78)	1	895	Right primary motor cortex
		9.1	11.6	(-36, -8, 56)	1	864	left primary motor cortex
		10.6	26.5	(16, -24, 78)	1	3212	Right premotor cortex
		9.1	23.1	(-30,-30, 76)	1	2636	left premotor cortex
		8	16.4	(10, -12, 72)	1	1569	Supplementary motor cortex
		12.1	40.7	(-28,-20, 80)	1	6106	Precentral gyrus
		9.64	22.2	(-44,-44,-10)	2	2540	Corpus callosum
	12	3.66	2.81	(-60,-12,18)	2	129	left primary motor cortex
		3.53	1.42	(14, 0, 46)	1	70	Right premotor cortex
		4.36	3.21	(-50, 6, 42)	2	164	left premotor cortex
		2.03	3.63	(-6, -2, 52)	2	84	Supplementary motor cortex
		4.36	4.62	(-5, 6, 42)	3	282	Precentral gyrus
		4.39	8.3	(-12,20,14)	5	549	Corpus callosum
	13	5.34	7.64	(24,-24,64)	1	446	Right primary motor cortex
		1.39	3.7	(-42,-20,46)	1	53	left primary motor cortex

Table 3: Mean and standard deviation of pre- and post-treatment of clinical characteristics of walking capacity and percentage of improvements

Group	Measured parameter	**10MWT**		**TUG**		**6MWT**	
		M	SD	M	SD	M	SD
Study	Pre	13.935	8.44	24.113	26.08	202	69.35
	Post	8.875	3.42	15.145	5.79	261.5	46.69
	% Improvement	**-36.3%**	**-59.4%**	**-37.2%**	**-77.7%**	**29.5%**	**-32.6%**
Control	Pre	10.5	4.52	17.511	6.18	203.66	55.34
	Post	7.88	3.07	14.573	6.05	222.83	79.70
	% Improvement	**-24.9 %**	**-32 %**	**-16.8%**	**-2.1%**	**9.4%**	**44%**

Figure 1: Anti-gravity treadmill training device. [51]

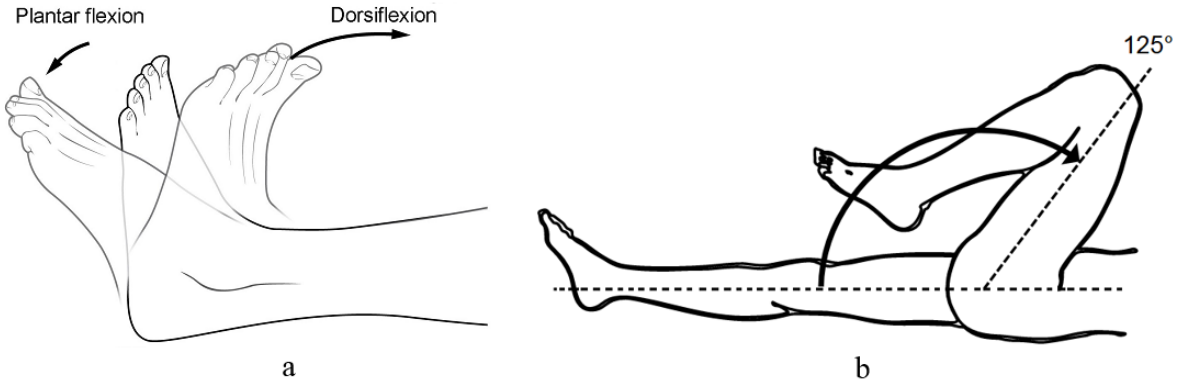

Figure 2: Passive tasks: a) Ankle plantarflexion to dorsiflexion over the range of motion (ROM), and b) Knee flexion to extension over the ROM.

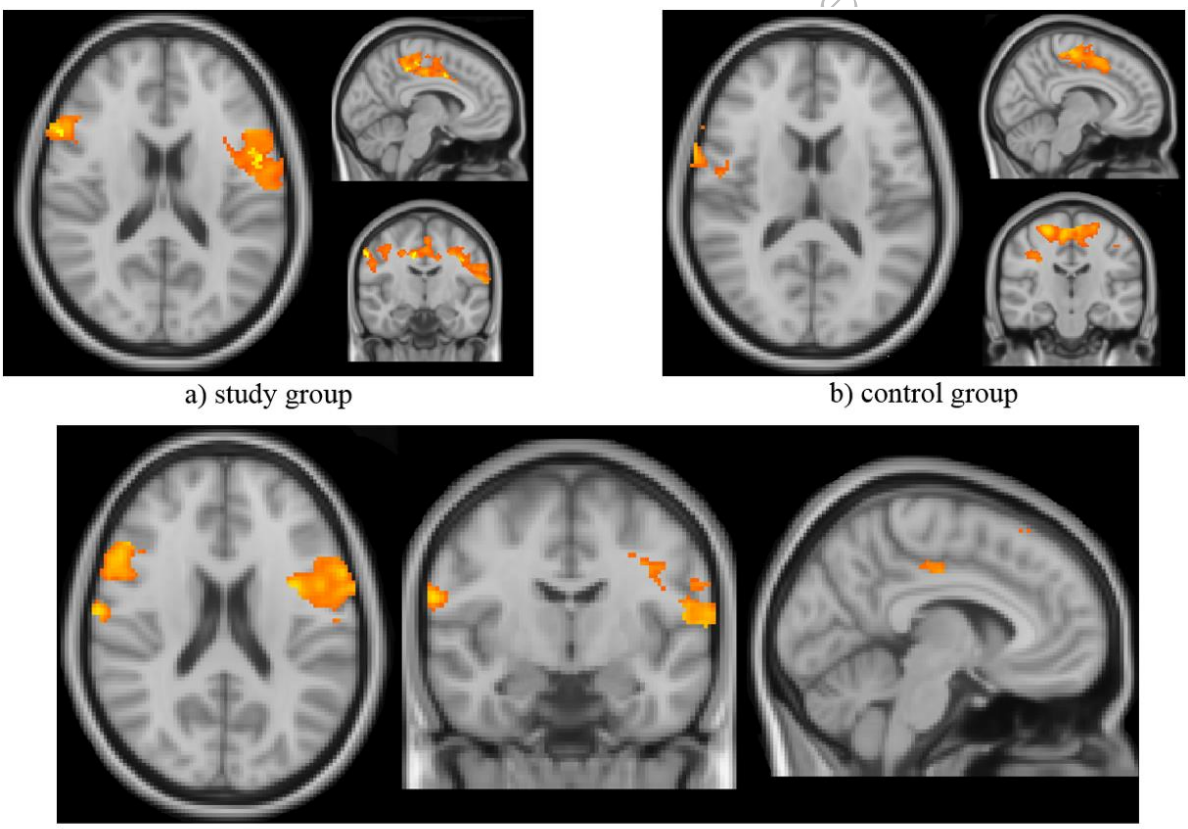

Figure 3: Significant difference in activation between pre-and post-treatment; a) study group, b) control group, c) significant differences in the average group results of the study and control groups. The red-yellow areas indicate voxel clusters with significantly higher brain activation at $p < 0.05$ (FDR corrections were utilized).