Title: Corpus Callosum Response Associates with Walking Capacity Improvement Following Treatments in Children with Cerebral Palsy: A Pilot fMRI Study

Running Title: Treatment-Induced Changes in Children with CP

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Abstract

Introduction: Gait impairment is one of the consequences of cerebral palsy (CP) as a permanent neurological disorder. In order to have persistent treatment effect, the therapy-induced neuroplasticity should have accompanied by functional improvement. In this regard, we aimed to determine the correlation between brain functional activity changes and walking capacity improvement following treatments in children with hemiplegic CP (HCP).

Method: Twenty-one spastic HCP children (7-12 years old) were randomly divided into 3 groups. Occupational therapy (OT) was performed for the first group. The second group received repetitive transcranial magnetic stimulation (rTMS) treatment and the third group underwent anti-gravity treadmill (AlterG) training. OT and AlterG training were provided for 45min, and rTMS was applied for 20min per sessions, 3 times per week for 8 weeks.

Brain functional activity while execution of passive tasks involving knee flexion/extension and ankle plantarflexion/dorsiflexion over the range of motion (ROM) was quantified using functional magnetic resonance imaging (fMRI). Walking capacity was indexed by clinical measures. Clinical and fMRI evaluations were performed pre- and post-training.

Result: Our results indicate that intensive OT, rTMS and AlterG training enhanced brain functional activity as well as walking capacity in pediatrics with CP. Also our results revealed a robust correlation between the corpus callosum functional activity changes and walking capacity improvement.

Conclusion: Regardless of the type of treatment, the improvement in gait function in children with CP may reflect as an increase in brain functional activity in callosal neurons which in turn may reveal the increase in interhemispheric coupling.

Keywords: Functional magnetic resonance imaging (fMRI), Cerebral palsy (CP), Correlation, Corpus callosum (CC), Gait

Highlights:

- Task-based fMRI can be considered as an effective tool for capturing brain functional activity changes induced by therapeutic interventions in children with CP.
- Intensive OT, rTMS and AlterG training enhanced both brain functional activity and walking capacity in children with CP.
- Regardless to the type of treatment, the improvement in clinical measurements have a robust correlation with corpus callosum functional activity

1. Introduction

Functional deficit including gait impairment in childhood is mostly one of the consequences of cerebral palsy (CP), a permanent neurological disorder, with the prevalence of 2-3 per 1000 live birth which results from a lesion or insult to the developing brain in early life [1–3]. CP as a non-progressive brain disorder results in impaired gait and posture which in turn affect the developing musculoskeletal system. Spastic hemiplegic CP (HCP) as a common subtype of CP is identified by unilateral involvement in the extremities [4,5].

Although medications including Botulinum toxin and orthopedic surgery have been used for children with CP, they have their own pros and cons including limited therapeutic efficiency and side effects to name but a few. Rehabilitations including occupational therapy (OT) and physical therapy (PT) are the most widespread treatment strategies for pediatrics with CP which focus on strengthening muscles and joints mostly in a passive manner [4,6]. However, efficient treatment should have accompanied by adequate voluntary long-term intensive systematic training. In this regard, anti-gravity treadmill (AlterG) provides the subject with the possibility of performing voluntarily gait and postural training. The AlterG can help individuals walk independently and actively using precise unweighting technology with adjustable speed. Successful treatment by triggering neural messaging in motor and sensory pathways is expected to cause brain neuroplasticity as well as forming or activation of specific neural pathways result in gait and postural improvement. [7–10].

Specific neural pathway formation also may have expected from repetitive transcranial magnetic stimulation (rTMS) which directly increase the activity and strengths of neural tracts. However, deploying rTMS in line with occupational therapy may increase its efficiency in improving gait and function [11–14].

Since cerebral palsy is a consequence of brain injury, improvement in brain structure and function in line with consistent clinical enhancement may result in long-lasting treatment. Considering this, other than clinical assessment following treatment, investigation of therapy-induced neuroplasticity is of great value. Although both neural and functional assessment explore the therapy efficacy, the former may further

provide evidence for lastingness of the treatment effects in the case of association between investigated neuroplasticity and improved function following treatment.

In this regard, advanced neuroimaging modalities such as structural magnetic resonance imaging (MRI), functional MRI (fMRI) and task-based fMRI (t-fMRI), are used for neuroplasticity assessment [7,15–20]. The latter is used to characterize functionally involved brain regions while performing a specific task [18,21–23]. T-fMRI provides hundreds of thousands of signals, reflecting functional brain activity, resulting from each voxel of the brain [13,24–26]. This helps us understand how the human brain is functionally organized into distinct subdivisions. Due to technical challenges in acquiring t-fMRI in children with spastic HCP, to date a limited number of studies have used this technique to explore gait rehabilitation-induced neuroplasticity in pediatrics with spastic HCP [25,27]. Also, some small-scale studies have detected fMRI alternations following therapy in children with unilateral CP [25,26].

The feasibility of standard processing steps of fMRI is mostly restricted by motion artifacts in children with CP as well as studies on healthy children, fMRI acquisition necessitates subjects remaining fixed for a long time [13,25,26]. For reducing head motions while imaging, sedation with least impact on administrated motor and sensory stimulation is used [28,29]. Previous studies also yielding reasonable results performing passive motor tasks during sedative fMRI [30–33].

Investigation the motor cortex as the source of most of descending motor pathways and the corpus callosum (CC) as the largest interhemispheric commissure is of great interest in literature. In case of prematurely born children or pediatrics with periventricular leukomalacia, motor performance has been shown to positively correlated with CC size [34–36]. CC as an extensive inter-hemispheric communication line play a crucial role for motor control [37]. Reid et al. showed that poor gross motor function classification system (GMFCS) is related to severe axonal loss of CC measured by MRI [38]. Also Peterson et al. showed that postural control enhancement is associated with structural integrity of CC in patients with multiple sclerosis (MS) [39]. Although to date fMRI studies mostly investigated gray matter, recent studies

also explored the functional organization of CC using t-fMRI [40,41]. However, the effect of treatment on CC and motor cortex functional activities still remains elusive.

We therefore aimed to explore the effects of OT, rTMS, and AlterG training on walking capacity as well as on brain functional activities of motor cortex and CC in children with CP, and to determine the possible associations between these measurements in this pilot study.

2. Materials and Methods

2.1 Participants

Twenty-one hemiplegic CP children (7-12 years old) with level II and level III GMFCS participated. They were cooperative and capable of following instructions; the participants had no severe cognitive impairment, no botulinum toxin injections within 3 months of the study, no previous constraint therapy within 9 months of the study and no history of orthopedic surgery 6 months prior to training. Two subjects did not participate in the fMRI portion of the study and one fMRI data was excluded due to excessive head motion.

2.2 Treatment Protocols

The participants were randomly divided into 3 groups; OT, rTMS, and AlterG. All participants were provided with 24 sessions of treatment. Schematic diagram of this study is shown in Figure1.

2.2.1 Occupational Therapy

OT training which provided by a pediatric occupational therapist concentrated on reduce spasticity, gait training, coordination of agonist and antagonist, protective balance exercise, active-passive range of motion (ROM) and foot-eye coordination. Each participant received 24 session of 45-minute of OT 3 days/week.

2.2.2 Repetitive Transcranial Magnetic Stimulation

RTMS as a non-invasive multisession treatment technique deploys magnetic fields to stimulate nerve cells of cerebral cortex by altering cortical plasticity [42]. rTMS refers to the application of recurring TMS pulses to a specific brain region and was performed by "MagStim Rapid^2" [43] and its D70 air film coil

(AFC). Depending on the rTMS frequency, it could increase or decrease cortical excitability by applying high-frequency ($\geq 5Hz$), or low-frequency ($\leq 1Hz$), respectively. The former may have excitatory effect on brain function and increased motor function, while the latter could suppress the brain function. Due to the risk of seizure initiation in high-frequency rTMS, we applied low-frequency rTMS on contra-lesional primary motor cortex (M1) to inhibit the unaffected motor cortex excitability which may indirectly force the affected hemisphere to enhance its function. For positioning the rTMS coil over the stimulation targeted site Brainsight system, compatible with MagStim, is used. To prevent head movement, the head position was fixed using the device's equipment (Figure 2a). The resting motor threshold (rMT) has been defined as the minimum intensity stimulus necessary to drive motor evoked potentials (MEP) in the resting target muscle during single pulse TMS (50 μV(of peak-to-peak MEP amplitude occurs in 50% of 10 stimulations). To acquire rMT in each participant, electromyography (EMG) active electrodes were placed on the unaffected leg to determine MEP of the tibialis anterior (TA) relaxed muscle during single pulse TMS with same treatment coil. These resting motor thresholds were used to adjust the intensity of rTMS for each participant. 1200 rTMS pulses in the form of 120 trains with an inter-train interval of 1 second was applied to each subject for 24 sessions, 4 days/week [44]. From the 13th session the rTMS was followed by 45 minutes of OT.

2.2.3 Anti-Gravity Treadmill Training

The anti-gravity treadmill (AlterG F320, CA USA) apply positive air pressure into an inflatable chamber that surrounds the lower extremities to uniformly reduce gravitational load and unweight the participant to 20% of his body weight gradually in 1% increments (Figure 2b). The patients received 45-minutes AlterG training 3days/week for 8 weeks. In each session, participants initiated their walking with the speed of 1km/h while they were unweighted to 50% of their body weight to warm up. As the speed increased, the body weight support gradually decreased based on the patients walking capacity on the treadmill and also discretion of the trained physical therapist who provide the participants with necessary feedbacks [7–9].

2.3 fMRI Acquisition

Scanning was performed with a single-shot gradient echo-planar imaging (EPI) pulse sequence and standard head coil using a GE 3-Tesla scanner. Eighty axial slices parallel to the posterior/anterior commissure line with a slice thickness of 3mm were acquired using a repetition time (TR) of 3s, echo time (TE) of 30ms, a flip angle of 90° and 64×64 matrix size. Following completion of the fMRI protocol a T1-weighted image was acquired parallel to the posterior/anterior commissure line (resolution=1×1×1 mm, TR=1800ms, TE=3.4ms, matrix size=256×256).

Each participant underwent MRI scans pre- and post-treatments. Pediatric anesthesiologist sedated the participants prior to MRI data acquisition. The default protocol for sedation involved intra-venous Propofol administered at the lowest dose to keep the patient asleep after induction. Throughout the examination, heart rate and oxygen saturation were monitored for all subjects by pulse-oximetry. Information about the sedation was documented in the medical record.

Knee flexion/extension and ankle plantarflexion/dorsiflexion movements over the range of motion (ROM) with 0.5Hz and 1Hz frequency respectively, were defined as two passive motor tasks for acquiring t-fMRI. A trained biomedical engineer performed all passive tasks. A block design of 24s of rest alternating 24s of motor task repeated for 5 cycles was used for fMRI acquisition

2.4 FMRI Data Processing

Statistical analysis and pre-processing of the functional data were performed with fMRI of the brain (FMRIB) software library (FSL v6.02). Brain extraction, realignment, spatial smoothing, motion correction, denoising and filtering as the standard pre-processing steps were applied on fMRI raw data. The brain images were coregistered and realigned to the mean functional image from the first session. The realigned images are skull-stripped with brain extraction tool (BET) in FSL. To reduce motion artefacts, the subjects' motion parameters were extracted and included as nuisance covariates in individual analysis. Then the functional images were smoothed with a $5\times5\times5mm^3$ full width at the half maximum (FWHM) Gaussian kernel and a high pass (HP) filter of 72s. In order to enhance the signal to noise ratio (SNR), spatial smoothing was deployed. Voxels resides exterior to the brain were removed. For denoising,

multivariate exploratory linear optimized decomposition into independent components (MELODIC) was performed. MELODIC uses independent component analysis (ICA) to decompose a 4D data sets into different temporal and spatial components [45,46].

FMRI data transformation into standard space may affect group difference as well as brain functional analysis outcomes. Hence, the reverse process of registering Montreal neurological institute (MNI152 atlas) standard space to brain functional data is performed [47]. This study uses region of interest (ROI) analysis to cover the motor cortex and corpus callosum (CC). Motor cortex includes brain regions related to planning (premotor cortex (PMC)), execution (supplementary motor area (SMA) and precentral gyrus (PG)) and control of movements (primary motor cortex (M1)). These regions were selected from the Harvard-Oxford subcortical and cortical atlases, and subsequently transformed to the individual's native space.

To calculate the significant brain regions, first-level individual statistical analyses were performed using the general linear model (GLM). To estimate brain activation for the separate contrasts (rest < passive movements), second-level random effects (RE) models were used for each group. Then t-test was used to detect significant differences in pre-treatment compared to post-treatment for rest < passive movements contrast. All contrasts were reported for false discovery rate (FDR), $p < 0.05$ and clusters comprising at least 10 voxels.

2.5 Clinical Evaluations

In this study 3 common clinical characteristic used to evaluate balance and movement including 6-minute walk test (6MWT), timed-up-and-go (TUG) and 10-meter walk test (10MWT). 6MWT evaluate person's walking endurance by measuring the distance walked in 6 minutes [48]. TUG assess both static and dynamic balance by measuring the time required for subsequently performing the following tasks: rising from a chair, walking 3 meters, making a U-turn, walking back 3 meters to the starting point and siting down on the chair while turning 180° [49]. 10MWT assess functional mobility and gait by measuring walking speed of a 10-m walk at the self-selected velocity (SSV) and fast velocity (FV) [50].

3. Results

3.1 Therapeutic Effects on Gait and Balance Impairment

Table 1 summarizes the percentage of clinical characteristics improvements following 24-sessions of training course for each subject. Clinical characteristics includes TUG, 6MWT and 10MWT. In OT group TUG, 6MWT and SSV improved in all subjects and FV improved in all subjects except one. In rTMS group TUG improved in all subjects while 6MWT and 10MWT improved in all subjects but one and two cases, respectively. In AlterG group all clinical parameters improved in all subjects.

3.2 Therapeutic Effects on Functional Brain Activity

In this study, the functional activation investigated in 2 brain areas: motor cortex and CC which connects two hemispheres. The motor cortex includes brain regions associated with planning (PMC), execution (SMA and PG) and control of movements (M1). Table 1 summarizes significant difference between pre- and post-treatment in motor cortex and CC in terms of the number of activated voxels for all tasks and each subject. According to the Table 1, in each group the number of activated voxels in CC decreased in one subject and increased in others due to treatments. In motor cortex, the number of activated voxels is a combination of increase and decrease following treatments.

Figure 4 depicts significant difference in CC activation between pre-treatment and post-treatment for all subjects and p-value less than 0.05 (FDR corrections were applied).

3.3. Correlation Results

According to the Table 1 the difference in brain functional activity between pre- and post-treatment in motor cortex is negative in 10 participants while is positive in the others. Although in 6 subjects the clinical characteristics are improved following treatment, the number of activated voxels decreased in motor cortex. Since the decreased in cortical activation may not represent the deterioration in functional activity [51], the absolute value of motor cortex functional activity changes used for investigation the possible correlation with clinical parameters improvements in Figure 3. In rTMS and OT groups, motor cortex functional activity has no correlation with clinical parameters. In AlterG group, motor area functional activity

significantly correlates only with FV (p-value < 0.05). For all subject regardless to type of treatment, there is no significant correlation between motor cortex activity and clinical parameters (Figure 3).

Figure 3 also shows the correlations between clinical improvements and the value of the functional activity changes in corpus callosum. In all groups, FV changes following treatment significantly correlates with CC functional activity alternations. However, SSV and 6MWT changes only significantly correlate with brain activity changes in CC region following rTMS and AlterG training, respectively. Figure 5 shows the CC functional activity alternations and FV changes for all subjects.

4. Discussion

In this pilot study we explore the therapeutic effects of intensive OT, rTMS and AlterG training on walking capacity and induced neuroplasticity indexed by brain activation alternations using passive task-based fMRI. Passive tasks include plantar flexion and dorsiflexion over the ROM of both ankles as well as flexion to extension over ROM of both knees, which were carried out pre- and post-treatments under sedation. To our knowledge, limited number of studies investigated brain functional reorganization following gait rehabilitation in pediatrics with cerebral palsy and adults with stroke [25,52,53]. The studies that utilized OT and Lokomat for walking capacity enhancement, mostly focused on clinical improvement rather than brain reorganization assessment [54–56]. Furthermore, the effect of treatment on interhemispheric relation and brain functional activities remains elusive. Therefore, we aimed to characterize the brain functional activity alternations following these treatments in children with spastic HCP in addition to assessment of walking capacity improvement. Also we aimed to investigate the neural correlation of clinical improvement in terms of brain functional activity changes in specific brain regions in children with CP induced by the treatments, regardless of type of treatment. Our findings showed an improvement in both walking capacity and brain functional activity following OT, rTMS and AlterG trainings. Also our result showed the improvement in clinical measurements is significantly correlated with brain functional activity changes in selected regions.

Motor cortex is responsible for planning, execution and control of movements and CC is considered to be the largest WM tract connecting the two hemisphere which is critical for performing tasks that require interhemispheric interaction. Moreover, the homotopic connectivity between the motor cortices is believed to emerge through CC. The interhemispheric exchange role of CC could be inhibitory, excitatory or combination of both which may alter though neurological diseases such as stroke and CP [57]. Additionally, positive correlation of CC of children with CP and their motor performance has been reported in previous studies [34–36]. In this regard, assessments of interhemispheric relations through CC may unravel the neurophysiological underpinnings that modulating motor control and may result in introducing interventional therapies to improve motor function in children with CP. Although to date number of studies investigates the activation of CC following different visual, motor and tactile tasks using fMRI, none of which explore its therapy-driven alternations. Addressing this issue and taking the lesion heterogeneity in terms of location and size in our CP cohort into account, this pilot study for the first time investigate the therapy-induced brain functional activity alternation in motor cortex and CC regions.

Our analysis showed both a decrease and increase in brain activation after therapy. According to the Table 1 in 6 subjects the clinical characteristics are improved following treatments while the number of activated voxels decreased in motor cortex. This decrease in cortical activation may be an evidence for less neural energy consumption following intensive training and thought as a gain in neural efficiency [51]. Furthermore, learning of specific tasks following intensive repetitive training, may allow the tasks to preform through memory-based processing by reorganizing the cortical representations of sensorimotor features which have been suggested for children with CP [58]. In line with our results, in a related study intensive motor training resulted in both decrease and increase in cortical hemodynamic response during task execution [59]. Also the cortical activation of the sensorimotor area following treatment has been reported to decrease in some studies, while others showed increased cortical activation [55,60]. However, limits to our current knowledge of neuroplasticity mechanisms, the interpretation of such alternations in cortical activity of the brain regions of interest is challenging.

As illustrated in Figure 3 alternations in hemodynamic response of motor cortex only correlates with FV in AlterG group. However, absence of these correlations is not equivalent to lack of neural associations. The reasons for this stem mostly from the complex and non-linear association between the underlying mechanisms of these measures which will be investigated in future studies.

On the other hand, according to the Figure 3, alternations in CC functional activity in OT and rTMS groups significantly correlate with functional mobility and gait improvement indexed by FV. In AlterG group changes in CC functional activity significantly correlates with both enhancements in functional mobility, gait and walking endurance indexed by FV and 6MWT, respectively. Regardless of the type of treatment, CC functional activity changes significantly correlate with alternations of TUG and 6MWT.

Also our analysis revealed a robust significant correlation between alternations in CC functional activity and walking capacity improvement in terms of fast velocity, regardless of treatment group. Figure 5 illustrates the point. This robust correlation is in line with the studies which have explained that the modulation of interhemispheric communications associates with the ability of the brain to develop new neuronal interconnections, compensate for impairments and acquire new functions, which refers to neural plasticity [57,61]. In children with CP, the less affected side of the brain tries to compensate for the weakness of the more affected side, and this amount of interaction can be mainly related to the relationship between the two hemispheres.

5. Limitation

Our study has some limitations. Firstly, some subjects could not complete the required training sessions due to the intensive training schedule. Secondly, few fMRI data were not used due to excessive motion. Thirdly, despite the damping effect of sedation on brain activity, we successfully detect the brain activity and its changes following treatments. However, in practice brain activity changes following treatment must be greater than the ones reported in this study. Finally, characterization of the intervention effects may not solely be achieved by the calculation of the average group results and pre-post analyses. This required further time points and a larger sample size which is taken into consideration in our ongoing studies.

6. Conclusion

Our results indicate that intensive OT, rTMS and AlterG training enhanced both brain activity and walking capacity in children with CP. OT, rTMS and AlterG improve functional mobility and gait, while AlterG improves walking endurance as well. Regardless of the type of treatment, robust correlation between walking capacity improvement (FV) and CC activity changes were successfully characterized in children with hemiplegic CP. These results show that the improvement in gait function in children with CP may reflect as an increase in brain functional activity in callosal neurons which in turn may reveal the increase in interhemispheric coupling.

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8. Authors' Contributions

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

Conflicts of interest: The authors expressed no conflicts of interest.

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: Significant difference in activation between pre- and post-treatment in term of number of active voxels in CC and MC areas, and the percentage of clinical characteristics improvements (CC: corpus callosum, MC: motor cortex, TUG: timed-up and go, 6MWT: six-minute walk test, 10MWT: 10-meter walk test, SSV: self-selected velocity, FV: fast velocity)

						10MWT	
Subject		**CC**	**MC**	**TUG**	**6MWT**	**SSV**	**FV**
1	OT	3364	-280	12.4	4.6	50.5	61.2
2		1502	-1040	14	10	12.3	18.3
3		937	-432	2.1	7.1	5.4	5.6
4		-257	-1258	19.1	28.8	33.5	-9.6
5		1443	679	30.4	5.5	30.7	14.3
6		1341	-10744	38.65	20.5	45.3	38
7	rTMS	721	62	27.1	17.1	2.3	17.6
8		2421	8521	32.9	4.2	42.38	51.39
9		589	-794	51.1	37	41.64	28.7
10		-1270	-5326	13.84	12.5	-33.6	-8.79
11		3122	-9378	49.24	-8.6	10.3	70.8
12		357	-172	18.6	2.64	-9.2	17.14
13	AlterG	-293	61	21.6	6.6	20.7	10
14		1698	2263	10.5	35.1	24.4	21.5
15		83	426	13.7	15.3	2.8	12.7
16		51	2018	3.8	4	14.8	14.6
17		2190	-4296	11	20.5	58.8	57.5
18		4292	2015	70.7	220	46.7	69.1

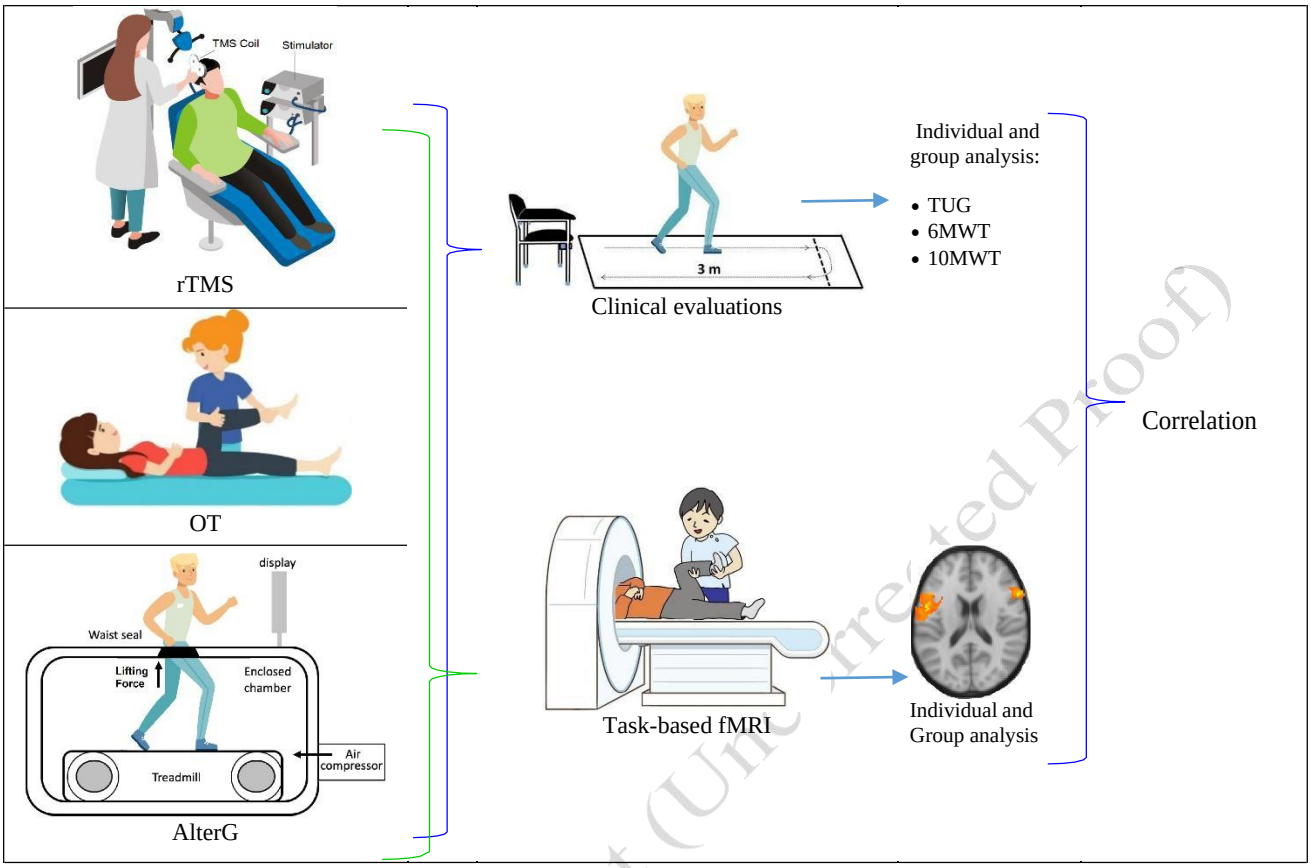

Figure 1: Schematic of the training and evaluation of children with CP

a B

Figure 2: a) Repetitive transcranial magnetic stimulation (rTMS).
b) Anti-gravity treadmill training device (AlterG)

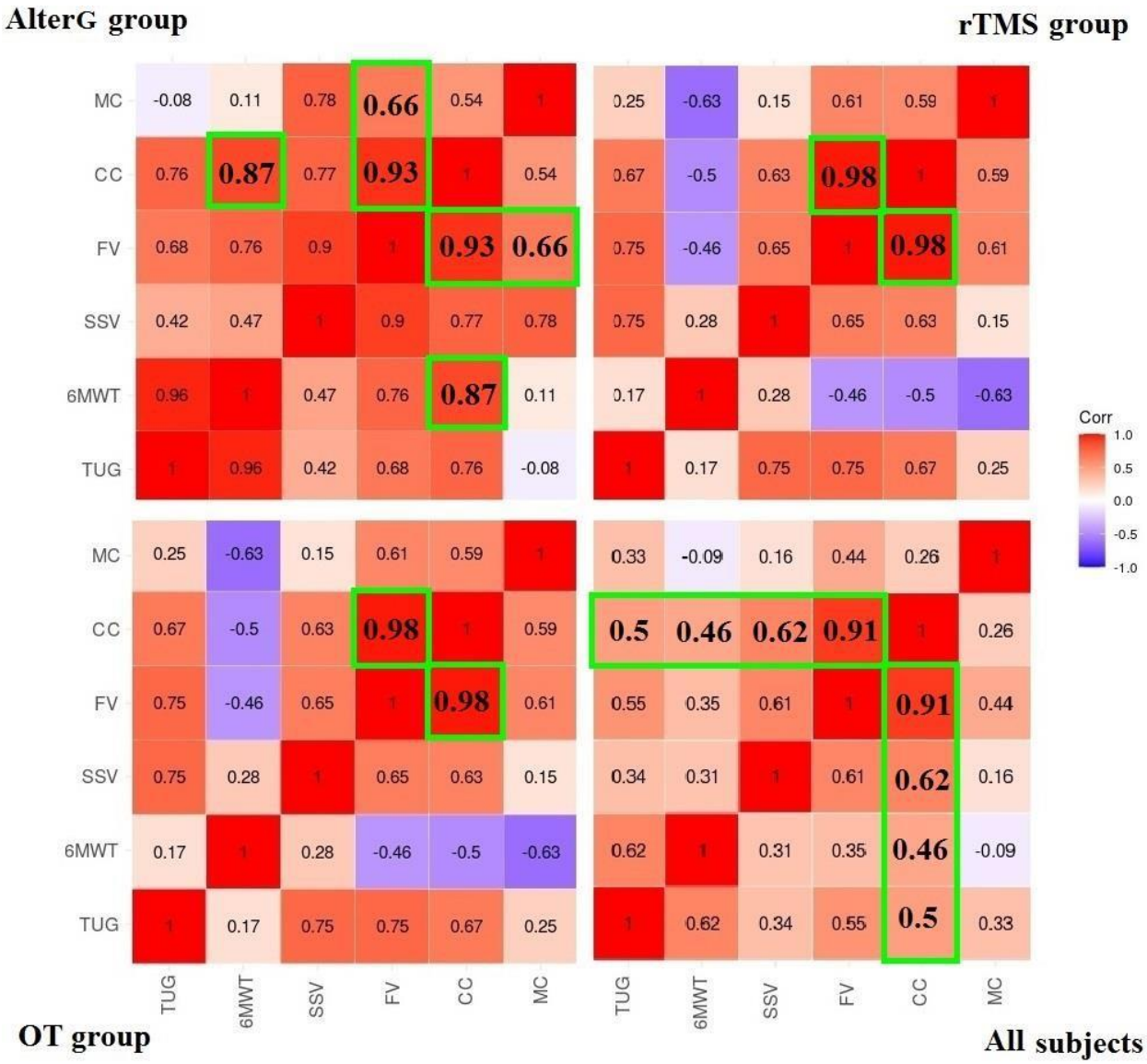

Figure 3: Correlations between clinical parameters improvements and the value of the change in CC and MC functional activity. The selected correlations are significant at $p<0.05$.
(CC: corpus callosum, MC: motor cortex, TUG: timed up and go, 6MWT: six-minute walk test, SSV: self-selected velocity, FV: fast velocity)

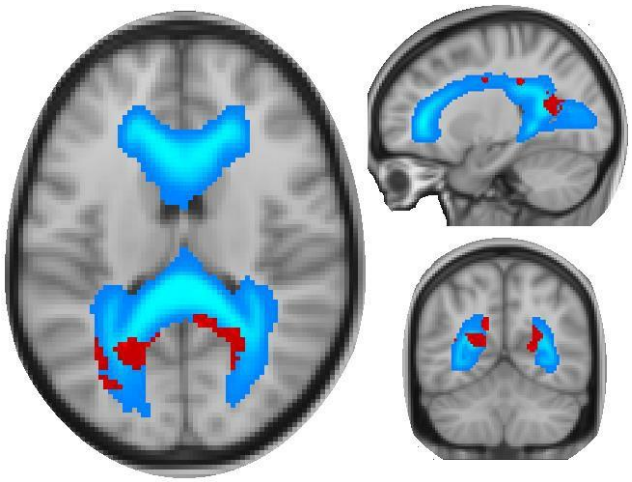

Figure 4: Significant difference in corpus callosum activation between pre-and post-treatment for all subjects, at $p < 0.05$ (FDR corrections were utilized)

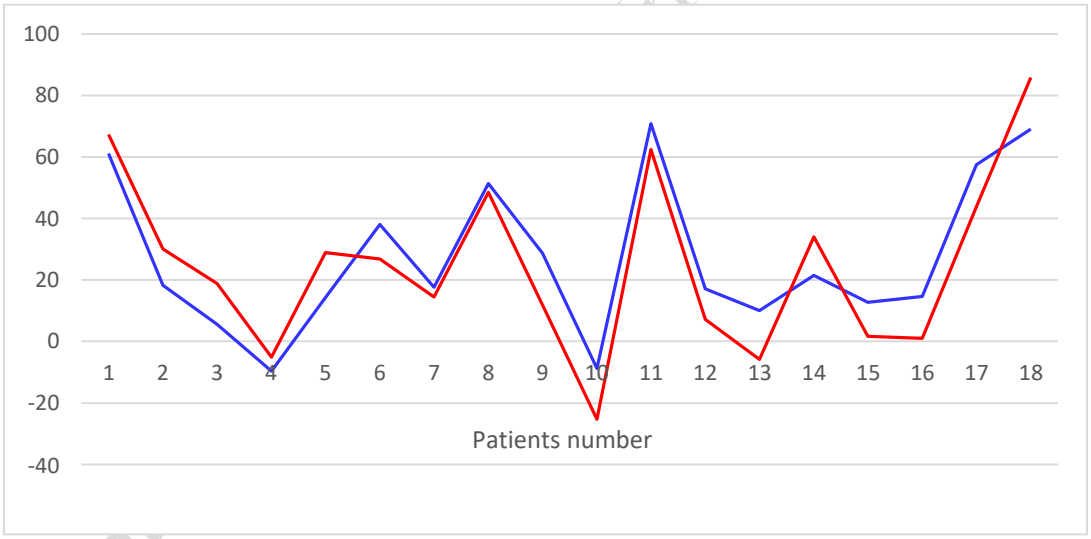

Figure 5: The red line is normalized difference in activation between pre- and post-treatment in term of number of active voxels in corpus callosum (CC), and the blue line is the fast velocity improvement for all subjects