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Identification of functional cortical plasticity in children with cerebral palsy associated to robotic-assisted gait training: an fNIRS study

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Abstract: Cerebral palsy (CP) is a non-progressive neurologic condition that causes gait limitations, spasticity, and impaired balance and coordination. Robotic-assisted gait training (RAGT) has become a common rehabilitation tool employed to improve the gait pattern of people with neurological impairments. However, few studies have demonstrated the effectiveness of RAGT in children with CP and its neurological effects through portable neuroimaging techniques, such as functional near-infrared spectroscopy (fNIRS). The aim of the study is to evaluate the neurophysiological processes elicited by RAGT in children with CP through fNIRS, which was acquired during three sessions in one month. The repeated measure ANOVA was applied to the β-values delivered by the General Linear Model (GLM) analysis used for fNIRS data analysis, showing significant differences in the activation of both prefrontal cortex ($F(1.652, 6.606)=7.638$; $p=0.022$), and sensorimotor cortex ($F(1.294, 5.175)=11.92$; $p=0.014$) during the different RAGT sessions. In addition, a cross-validated Machine Learning (ML) framework was implemented to estimate the gross motor function measure (GMFM-88) from the GLM β-values, obtaining an estimation with correlation coefficient $r=0.78$. This approach can be used to tailor clinical treatment to each child, improving the effectiveness of rehabilitation for children with CP ~~and, consequently, their quality of life~~.

Keywords cerebral palsy, neurological disorders, robotic-assisted gait training (RAGT), near infrared spectroscopy (fNIRS), Machine Learning (ML), rehabilitation.

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1. Introduction

Cerebral palsy (CP) is a non-progressive neurologic condition arising from brain injury that occurs before cerebral development is complete (Cherni et al., 2020), and it is a leading cause of motor disability in children. In fact, it is estimated that the average frequency of CP is 2.08 per 1000 live births, but when considering only children born with a body weight below 1500 g, the frequency increases of 70 times with respect to children with a body weight over 2500 g at birth (Sadowska et al., 2020). CP provokes muscle weakness, spasticity, bone deformities and impaired balance and coordination. Children with CP often exhibit a decrease in walking speed, an increase in double support duration, and poor endurance, with an impairment in daily activities, community integration and quality of life (Cherni et al., 2020). the Gross Motor Function Classification System (GMFCS) is one of the most common and easy-to-use scale to

describe the severity of motor disorders of CP (~~Palisano et al., 2006~~). This scale evaluates the child's independence when performing basic motor functions, and classifies the patients into five classes, where class 1 includes individuals who can freely walk, whereas class 5 includes subjects who are not able to move on their own (Palisano et al., 2006).

Several pharmacological approaches (Blackman et al., 2018) and rehabilitative techniques have been developed (Novak et al., 2017) to improve the quality of life of CP patients and their social integration~~.~~. Robotic-assisted gait training (RAGT) has become a common rehabilitation tool employed to improve the gait pattern of people with neurological impairments (Schwartz and Meiner, 2015). In fact, it was demonstrated that passive movements of the limbs could activate the central pattern generators (CPG), i.e. gait centers in the spinal cord (Rossignol et al., 2006). Importantly, it was proved that passive activation of the legs on a treadmill could evoke similar locomotor activity also in patients with severe spinal cord injuries (Schwartz and Meiner, 2015). With respect to the conventional body weight support treadmill training methods, RAGT has many advantages. For instance, RAGT allows the early beginning of rehabilitation also in severe patients, administering higher intensity of training as compared to traditional rehabilitation. Moreover, RAGT forces the patients in a more physiological and reproducible gait patterns, providing the possibility to evaluate the patient's performances. Finally, RAGT produces benefits also on cardiopulmonary functionalities (Billinger et al., 2014).

Two typologies of RAGT devices are commercially available: end-effectors and exoskeletons. End-effectors are basically composed of a double crank and rocker gear system to apply forces to the distal segments of limbs, and the patient is guided during the gait by a servo-controlled motor. Exoskeleton-type devices are robotic orthosis combined with a harness-supported body weight system combined with a treadmill, and the patient's legs are guided by the robotic device following a preprogrammed gait pattern. Among the exoskeletons commercially available, the Lokomat® (Hocoma AG, Switzerland) is a device which supports the patient on a treadmill while a robotic apparatus facilitates the inter-limb coordination and gait timing. The device provides several degrees of body weight support and guidance, that could be modulated according to the patient's needs, ~~resulting~~ highly suitable and effective to treat CP children (Schmartz et al., 2011). In fact, it was demonstrated that the employment of the pediatric orthosis of the tool could significantly decrease muscle stiffness after a single session of RAGT (Schmartz et al., 2011).

Moreover, it is worth to note that RAGT can produce a higher spinal and brain neuroplasticity as compared to the conventional body weight support treadmill training methods (Schwartz and Meiner, 2015). Neuroplasticity is the ability of the central nervous system (CNS) to go through permanent structural and functional modification in response to internal and external stimuli (Grafman, 2000). This capability can be exploited in damaged brain, which adapts its functionalities in response to rehabilitation. Specifically, CP patients' brain could be able to recover the control of motor functions through the neuroplasticity mechanism induced by rehabilitation. Notably, CNS exhibits a higher plasticity at the earliest stages of its development; hence, rehabilitation of children with CP should be started early (Granild-Jensen et al., 2015; Novak et al., 2017).

Motor rehabilitation allows restoring the lost motor patterns or developing new patterns that can compensate for the irretrievably lost functions through enforced motor activity. In this perspective, measuring the brain activity during the RAGT could provide information about the effect of the therapy on neuroplasticity. To this aim, portable neuroimaging techniques are more suitable than the techniques requiring high constraints for the patients (e.g., functional Magnetic resonance, fMRI,

magnetoencephalography, MEG) (Gramigna et al., 2017). Particularly, functional near infrared spectroscopy (fNIRS) is an optical neuroimaging technique able to measure the variations of oxyhemoglobin (HbO) and deoxyhemoglobin (HHb) secondary to the cortical activations, exploiting the optical properties of these chromophores in the near infrared spectral range (Forcione et al., 2020; Pinti et al., 2020). The feasibility of the combination between fNIRS and motor rehabilitation in CP children has been already demonstrated. ~~For instance, Kurz et al., reported that children with CP had an increased amount of activation in the sensorimotor cortices and superior parietal lobule during gait with respect to typically developed children (Kurz et al., 2014). Chaudary and colleagues observed a bilateral dominance in the prefrontal cortex of healthy controls and an ipsilateral dominance in CP patients during a ball throwing task (Chaudhary et al., 2014). Concerning the use of fNIRS during RAGT, Van Hedel et al., evaluated the cortical activity through fNIRS in children with neurological disorders during walking both with the Andago (a device enabling over-ground walking with bodyweight support) and on a treadmill. They demonstrated that only a small proportion of the participants with neurological disorders shows typical hemodynamic responses during walking in Andago, in contrast with healthy controls. Moreover, they demonstrated high levels of acceptance of the fNIRS by children with neurological disorders during the motor rehabilitation.~~

The aim of this study was to evaluate the neurophysiological processes activated by RAGT, in addition to conventional therapy, using fNIRS in children with CP. The novelty of this study relies on the demonstration of the possibility to employ fNIRS during RAGT in children with CP to evaluate in vivo the neuroplasticity induced by the rehabilitation.

2. Materials and Methods

2.1 Particpants

Ten children with CP attending the Neurorehabilitation Unit "Gli Angeli di Padre Pio" of the Padre Pio Foundation and Rehabilitation Centers, San Giovanni Rotondo, Foggia, Italy., between November 2021 and April 2022 were enrolled in this study. Two of them dropped out as they were positive for SARS-CoV-2 during the training, so the final sample was composed of eight individuals. Table 1 summarizes the participants' characteristics.

Table 1 The quantitative variable age is expressed as mean ± standard deviation, whereas the qualitative variables as absolute frequencies.

Participants	8
Age (years)	9,88 ± 4,73
Affected side (n)	
Unilateral	1
Bilateral	7
Cerebral palsy subtype (n)	
Spastic	8
Dyskinetic	0
Ataxic	0
GMFCS level (n)	
I	1

II	0
III	1
IV	5
V	1

Inclusion criteria were: (1) children with CP aged between three and eighteen with a GMFCS level of I-V, (2) ability to communicate discomfort or pain, (3) understanding simple instructions.

Exclusion criteria included medical conditions potentially interfering with the locomotor training, or physical limitations for using the robotic device. In addition to all relevant criteria outlined in the Lokomat manufacturer's manual, children are excluded if they have: severe lower-extremity muscle contractures, hip instability/subluxation, Botulinum toxin-A (BTX-A) injections to lower limbs within the last 3 months, seizure disorder that is not controlled by medication, open skin lesions or vascular disorder of the lower limbs; or if patients are not able to co-operate or be positioned adequately within the Lokomat.

2.2 Clinical evaluation

The participants underwent 3 RAGT sessions per week, each session lasting about 30 minutes, for a total of 12 RAGT sessions. RAGT was administered with the biofeedback provided by the Lokomat. In addition to the RAGT, the participants received also the traditional therapy. In order to evaluate the modifications of the motor abilities of the participants, the following clinical scales were admistered before (T0) and at the end of the training (T2):

- Gross Motor Function Measure-88 (GMFM-88): it consists of 88 items in five dimensions: lying and rolling (GMFM-A); sitting (GMFM-B); crawling and kneeling (GMFM-C); standing (GMFM-D); and walking, running and jumping (GMFM-E) (Salavati et al., 2015).
- Gross Motor Function Classification System (GMFCS): it provides a method of describing the functional ability of children with CP in one of the five levels. Children in Level I can perform all the activities of their age-matched peers, albeit with some difficulty with speed, balance, and coordination; children in Level V have difficulty controlling their head and trunk posture in most positions and in achieving any voluntary control of movement (Morris and Bartlett, 2004)
- Modified Ashworth Scale (MAS): it was developed by Bryan Ashworth as a method of grading spasticity. The original Ashworth scale was a 5 points numerical scale that graded spasticity from 0 to 4, with 0 being no resistance and 4 being a limb rigid in flexion or extension. However, Bohannon and Smith modified the Ashworth scale by adding 1+ to the scale to increase sensitivity. Hence, MAS varies from 0 (no increase in muscle tone) to 4 (affected part(s) rigid in flexion or extension) (Meseguer-Henarejos et al., 2018).

Shapiro–Wilk's normality test was applied to check the normality of the clinical data distribution of the scores. Since the data did not meet the assumption of normality, the Wilcoxon signed-rank test was performed between the scores obtained during the different sessions (i.e. T0 and T2) to evaluate the improvement of the muscular tone and the level of spasticity in response to RAGT.

2.3 Experimental procedure

After the participants were harnessed to the Lokomat, they received the RAGT experimental protocol (Figure 1a). Particularly, a block paradigm was used: the children

were first asked to actively move during the RAGT for 30s, then they were invited to rest for other 30 s. The paradigm was provided in 10 blocks, as described in Figure 1b.

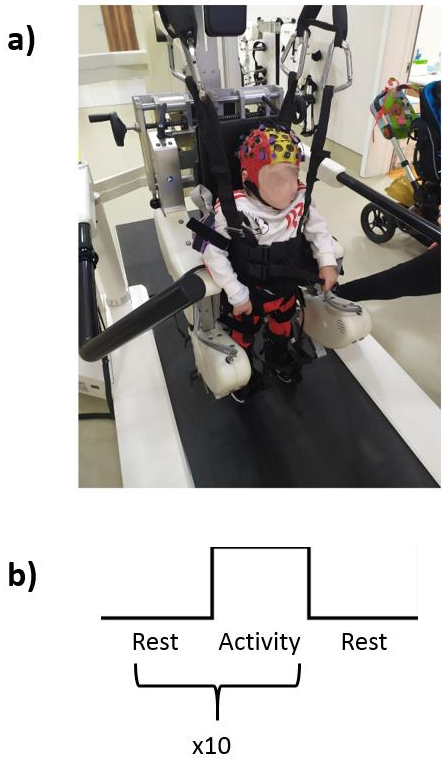


Figure 1 (a) Representative participant harnessed to the Lokomat (b) schematic block paradigm of the experiment.

2.4 fNIRS measurements and data analysis

To measure the cortical hemodynamic activity, the portable fNIRS Cortivision Photon cap device was used (the device was provided thorugh Cortivsion Pathfinder Program grant number CPP-2021/10/3). It is composed of 16 LEDs emitting at 760 nm and 850 nm wavelengths and 10 detectors. The montage delivered 34 channels covering the frontal, prefrontal and motor cortices, placing the optodes according to the 10-20 system. Moreover, 4 short channels have been provided in order to remove the physiological contaminations from the fNIRS signals (Brigadoi and Cooper, 2015). The fNIRS was recorded at the first session (T0), at the sixth (T1) and at the twelfth session (T2).

Source and detector locations were digitized using a Polhemus FastTrak 3D digitizer (Colchester, VT, USA; accuracy: 0.8 mm) composed of a recording stylus and three head-mounted receivers, which allowed corrections for small movements of the head in between measurements. Figure 2 shows the among subjects' average channels locations after warping of the digitized sources and detectors into MNI space (Colin27). According to a sensitivity analysis performed in NIRS-SPM (Ye et al., 2009), the brain Brodmann Areas (BAs) investigated were numbers 1, 4, 5, 6, 7, 8, 9, 10, 11, 40, 45 and 46.

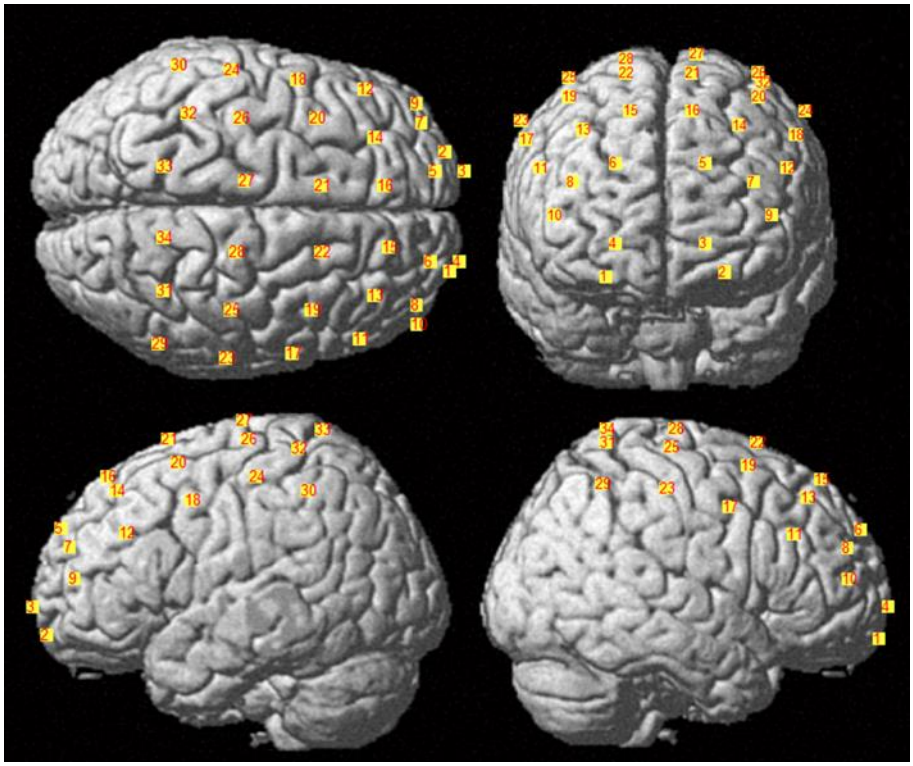


Figure 2 Average channels locations after warping of the digitized sources and detectors into MNI space

The fNIRS signals were converted into optical densities and then converted into HbO and HHb concentrations through the Modified-Lambert-Beer law. The DPF was computed for each participants in accordance with Scholkmann et al (Scholkmann and Wolf, 2013) and Chiarelli et al (Chiarelli et al., 2019). The hemoglobin signals were filtered with a zero-lag 3rd order Butterworth digital filter (cut-off frequencies 0.01-0.4 Hz) (Pinti et al., 2019) and the motion artifacts were removed through a Wavelet transform based algorithm (Perpetuini et al., 2021). Physiological contaminations have been corrected from the long separation channels through the procedure reported by Sato and colleagues (Sato et al., 2016).

The fNIRS data analysis was based on General Linear Model (GLM), which, in the matrix notation, is expressed as:

$$Y=X\beta+\varepsilon \quad (1)$$

where Y is a n × 1 column vector indicative of the considered time-series; X is a n × p design matrix, where each column corresponds to a predictor variable; β is a p × 1 column vector of the unknown weights of each predictor that indicate the strength of the association with Y; and ε is an n × 1 column vector associated to the residual error. The unknown parameter β is estimated through a least-square regression approach, providing information concerning the amount of variance of the signal explained by the predictor (Perpetuini et al., 2019). For fNIRS applications, the β-values are indeed indicative of the cortical activation and could be used for further statistical analysis. In order to assess statistical differences between the β-values at T0, T1 and T2, a one-way repeated measure ANOVA (RM-ANOVA) has been performed for each channel, where the within factor is the temporal session. In order multiple comparisons (paired t-test) were performed to assess which temporal recordings provided statistical differences, and, the results were Bonferroni corrected to avoid false positives, considering the number of channels and comparisons. This analysis was performed for both HbO and HHb.

Finally, a regression analysis based on Gaussian Process Regression was performed linking the cortical activations with the GMFM-88, to test whether the assessed changes in the brain activity were associated to modifications in the motor abilities. In detail, the β-values at T0 and T2 obtained from the GLM analysis (for both HbO and HHb) were used as input, whereas the GMFM-88 was used as output. Of note, a features selection based on Wrapper procedure (Kohavi and John, 1997) was performed to reduce eventual overfitting effects. A leave-one-subject-out cross validation was used to assess the generalization performances of the model.

3. Results

3.1. Clinical scales results

The results of the comparison of the clinical scales evaluated before (T0) and after the RAGT sessions (T2) are reported in Table 2. In particular, only the GMFM-88 showed a significant difference between T0 and T1 (T0 vs T1, z=-2.524; p=0,008), as shows in Figure 3.

Table 2 Median and IQR of clinical scale at baseline (T0) and post-treatment (T2). GMFM 88, Gross Motor Function Measure-88 total values; MAS H, Modified Ashworth Scale for hip muscles; MAS K, Modified Ashworth Scale for knee muscles; MAS A, Modified Ashworth Scale for ankle muscles

	(T0) Median (IQR)	(T1) Median (IQR)	p values
GMFM 88	16 (14 - 23.6)	24.3 (19.4 - 30.9)	z=-2.524; p=0,008
MAS H	1 (0.5-2)	1 (0.75-1)	n.s.
MAS K	2 (0.5-2)	1 (0-2)	n.s.
MAS A	2 (1-2)	1 (0.75-1)	n.s.

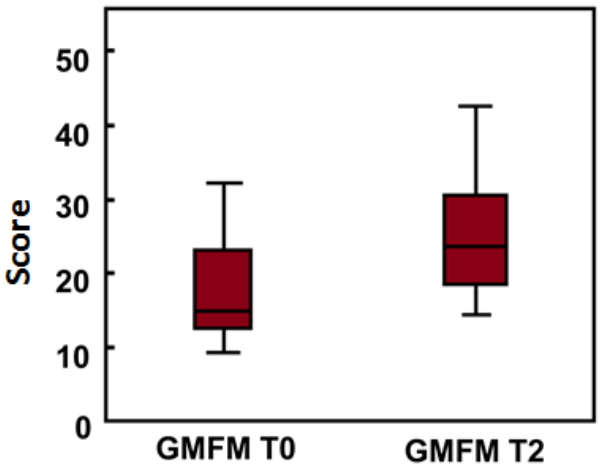


Figure 3 Boxplot associated to the GMFM-88 evaluated at T0 and T2

3.2 fNIRS results

Figure 4a reports the F-statistic map obtained performing the RM-ANOVA between T0, T1 and T2 for HbO signals, whereas Figure 4b reports the map obtained through the same comparison for the HHb signal. Notably, the figure highlights the channels that were still significant after the correction. Specifically, BA 9 and BA 11

showed significant differences bilaterally, whereas BA 6 and 45 showed a significant difference only for the left hemisphere, and BA 1 and 46 for the right one.

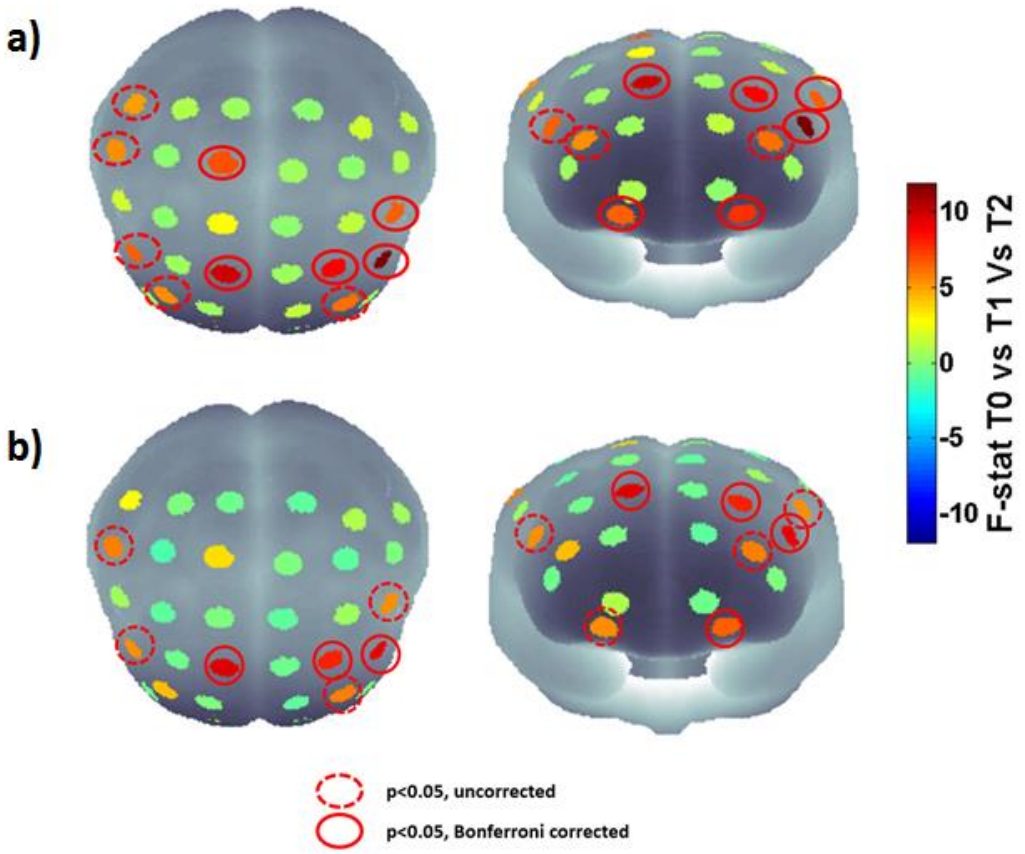


Figure 4 Statistical maps (F-statistic) comparing the cortical activation at T0, T1 and T2 for Hbo (a) and HHb (b). Dashed red circles shows significant channels ($p < 0.05$), that do not survive Bonferroni correction, whereas continuous red circles are associated to channels that are still significant ($p < 0.05$) after multiple comparison correction.

The significant post-hoc multiple comparisons results are reported in Table 3. Of note, significant differences, applying the Bonferroni correction, were obtained only between T0 and T2, except for the left frontal zone (corrected p-value: 0.04), which also showed differences between T0 and T1.

Table 3 Multiple comparisons (paired t-test) associated to the RM-ANOVA. Only significant comparisons were reported in the table.

	Channels	Comparison	t-stat	Adjusted p-value
HbO	Ch1	T0 vs. T2	-3,747	0,020
	Ch2	T0 vs. T1	-3,799	0,018
	Ch12	T0 vs. T1	-3,788	0,019
	Ch12	T0 vs. T2	-4,181	0,014
	Ch14	T0 vs T2	5,937	0,004
	Ch15	T0 vs T2	4,756	0,009
	Ch18	T0 vs T2	-4,075	0,015
	Ch28	T0 vs T2	-4,037	0,016

HHb	Ch2	T0 vs. T1	-3,083	0,036
	Ch12	T0 vs. T1	-3.471	0,026
	Ch12	T0 vs. T2	-4.093	0,014
	Ch14	T0 vs T2	5,099	0,007
	Ch15	T0 vs T2	4,056	0,015

Finally, the regression analysis demonstrated an association between the cortical activation modifications and the improvement of the motor abilities. In detail, the Wrapper procedure selected the channels 7, 12 and 14. This subset of features was used as input of the Gaussian Process Regression, which estimated the GMFM-88 with a correlation coefficient of 0.78 (p=0.001) and a root mean square error of 0.636, as reported in Figure 5.

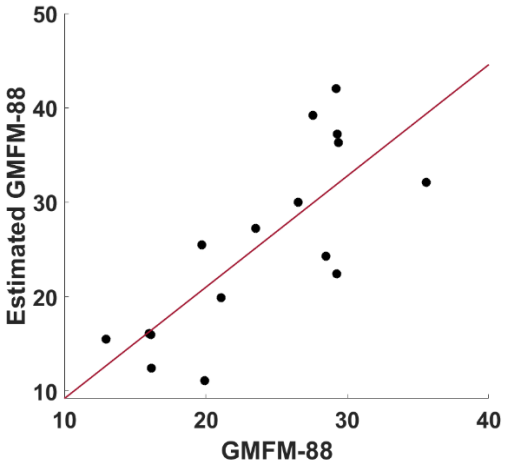


Figure 4 Scatter plot between the evaluated GMFM-88 and the estimated GMFM-88 obtained through a leave-one-subject-out cross-validated Gaussian Process Regression from fNIRS β-values selected employing a Wrapper procedure.

4. Discussion

The aim of this study was to assess the neurophysiological processes activated by RAGT in children with CP using fNIRS. The findings demonstrated bilateral modifications in the cortical activations in BA 1, 6, 9, 11 and 46. Specifically, the post-hoc analysis determined a decreased activation in BA 9 during the session and an increased activity of BA 1, 6, 11 and 46. In detail, BA 11-6-9 areas are involved in the motor movement sequence planning, reward, long-term memory, decision making. Moreover, BA 6 and 9 participates also in the control of the intentionality of movement, complex movements and coordination. BA 46 and 9 are also related to the attention, working memory, and self-control, whereas BA 1 is involved in proprioceptive and fractional movement control. Finally, BA 9 is related also to spatial memory (Strotzer, 2009). Notably, the multiple comparisons analysis delivered significant differences, FDR corrected, only between T0 and T2, except for the left BA 46, which shows differences also between T0 and T1. Importantly, these modifications in cortical activity are associated to improvements in the motor functions, as shown by the significant increase of the GMFM-88 and sub-scores. This finding is in line with previous studies demonstrating the benefits of the RAGT in diplegic children with CP. For instance, Wallard et al found a significant improvement of the kinematic data of the full-body in the sagittal and frontal planes and of the Gross Motor Function Measure test due to the

RAGT therapy administered through Hocoma Lokomat, demonstrating the usefulness of RAGT in improving the balance control in gait (Wallard et al., 2017). Van Kammen and colleagues found positive effects associated to the RAGT as well. In particular, they demonstrated that walking with the Lokomat reduces muscle activity in children with CP, whereas altering guidance or BWS generally does not affect amplitude (van Kammen et al., 2020). Nonetheless, these studies do not provide information regarding cerebral functioning modifications associated to RAGT and motor control improvements. In this perspective, it should be highlighted that several neurological diseases are associated to gait disorders whose neurofunctional correlates are scarcely investigated. To this aim, fNIRS is particularly suitable to study neurophysiological ~~modifications~~ during rehabilitation since it allows investigating functional brain hemodynamic oscillations in ecological settings, without hard constraints for the individual (Gramigna et al., 2017) and the present work constitutes a fundamental step forward the investigation of this neurophysiological aspects.

Concerning the employment of fNIRS during gait, Kurz et al. found that children with CP exhibit an increased activation in the sensorimotor cortices and superior parietal lobule during gait with respect to typical developed children. Moreover, they demonstrated that children with CP had a higher variability and number of errors in stride time intervals and temporal gait kinematics (Kurz et al., 2014). Although Kurz et al evaluated the cortical activity during walking on a treadmill (and not during RAGT), their findings could provide insights in the interpretation of the results of our study. In fact, the multiple comparison analysis showed a decreased cortical activation through the RAGT sessions in the BA 9, which is related to the motor planning and coordination, suggesting that this robotic training could help the brain to function in a manner similar to the typical developed children, and this is characterized by a reduced brain activity in the motor cortex.

The increased activation found in the prefrontal cortex could be associated to an increase of the attention and participation of the patients during the RAGT session. This hypothesis could be corroborated by the ML analysis. In fact, the developed ML framework evaluated the relationship between the hemoglobin oscillations and the modifications in the motor functions (GMFM-88). In order to reduce the risk of an overfitting effect, an automated features selection based on the Wrapper method was conducted. The procedure selected channels that covers the prefrontal and somatosensory cortex, showing a link between cortical activity modifications in those areas and the motor functions improvements due to RAGT. However, further studies are necessary to increase the sample size. In fact, since the fNIRS-based regression outcome relies on a multivariate analysis of all the channels, the regression performances might strongly increase enlarging the sample numerosity. However, it should be highlighted that, although the sample size could be considered small, the estimation was conducted applying a leave-one-out cross-validation procedure, that excludes one participant at a time and testing the classifier on that specific participant, thus evaluating the out-of-sample performance obtaining generalizable results.

These results could pave the way to the employment of the fNIRS in clinical practice with the aim of estimating the responsiveness of the children to the therapy in terms of neural plasticity and motor functions, allowing to modulate the treatment to the needs of the patients, thus increasing its effectiveness. These findings could also foster the establishment of shared guidelines regarding the administration of the RAGT. In fact, there are not standardized dosage and guidelines of the RAGT in CP. In fact, there are only two reviews describing respectively 486 CP patients in 17 studies (Lefmann et al., 2017) and 217 patients in 10 studies (Carvalho et al., 2017) undergoing robotic treatment. The data from these works show that the majority of the studies focus more children

with CP classified as ~~I-IV~~ GMFCS level I to IV, excluding more serious patients with GMFCS V level. Moreover, a heterogeneity in the choice of treatment protocol, whose duration varies from a minimum of 30 minutes to a maximum of 60 minutes, is described by the two reviews. Moreover, sessions range from 2 to 5 per week, repeated for 2-6 weeks, up to a maximum of 10 weeks as reported by Sarhan's et al. (Sarhan et al., 2014). Then, we suggest employing fNIRS in the clinical setting to standardize the RAGT protocols in children with CP, in order to avoid the heterogeneity of the clinical approach and maximize the neurological and motor benefits of the therapy.

It is noteworthy that our study shows that most of the changes in brain activity are found between T0 and T2, suggesting that a minimum number of sessions is needed to produce brain plasticity effects. However, it should be highlighted that the protocol was carried out 3 times a week for a total of 12 sessions. It would be interesting to verify if the same changes in the cortex would have occurred in an intensive daily training. Moreover, it should be highlighted that the present study was limited to a protocol of 12 sessions, but a medium and long-term follow-up would be necessary to investigate whether the modification of the cortical activity and the clinical improvements detected through GMFM 88 are definitively acquired by the children.

It should be stressed that the protocol excluded uncooperative children who were unable to follow the augmentative feedback presented on screen by the Lokomat. However, it should be investigated in future studies whether intensive RAGT could provide benefits also in non-cooperative children with severe cognitive deficits, who are normally excluded a priori from robotic rehabilitation protocols. As future perspectives, further studies comparing groups of children treated with RAGT and biofeedback with children undergoing RAGT only should be fostered. In addition, it would be interesting to compare a control group with CP children undergoing conventional gait training and RAGT.

5. Conclusions

This study is the first application of neuroimaging in children with CP during RAGT, providing information on the neural substrates related to the locomotor behaviors of children with CP. The results showed that robotic therapy produces modifications in brain activity in both the motor and frontal cortex with an improvement in motor control and attention during RAGT. This approach can be used to tailor clinical treatment, improving the effectiveness of rehabilitation for children with CP ~~and, consequently, their quality of life~~. This study could pave the way for further experiments aimed at monitoring and predicting the effectiveness of rehabilitation also in other pathologies with the aim of administering the most suitable therapy for each patient.

Author Contributions: Conceptualization, D.P., E.F.R., D.C., A.M., R.S.C., and S.F.; methodology, D.P., E.F.R., D.C. and S.F.; software, D.P.; validation D.P., E.F.R., and D.C.; formal analysis, D.P..; investigation, D.P., E.F.R., D.C., C.F., M.T., F.P., and G.P. D; resources, A.M., R.S.C., and S.F..; data curation, D.P., E.F.R., and D.C.,; writing—original draft preparation, D.P.; writing—review and editing, D.P.; visualization, , D.P., E.F.R., D.C., C.F., M.T., F.P., and G.P. D; supervision, A.M., R.S.C., and S.F.; project administration, A.M., R.S.C., and S.F.. All authors have read and agreed to the published version of the manuscript..

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

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy issues.

Conflicts of Interest: The authors declare no conflict of interest.

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