European Journal of Physical and Rehabilitation Medicine 2024 August;60(4):643-55
DOI: 10.23736/S1973-9087.24.08413-2

ORIGINAL ARTICLE

# Effectiveness of action observation treatment based on pathological model in hemiplegic children: a randomized-controlled trial

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## ABSTRACT

BACKGROUND: Action observation treatment (AOT) is an innovative therapeutic approach consisting in the observation of actions followed by their subsequent repetition. The standard version of AOT consists in the observation/imitation of a typically developed individual, which is proposed as model (TDM-AOT).
AIM: This study aims to compare the effectiveness of AOT based on a pathological ameliorative model (PAM-AOT) *versus* TDM-AOT in improving upper limb ability in children with unilateral cerebral palsy (UCP).
DESIGN: The study consists in a prospective randomized controlled, evaluator-blinded trial (RCT), with two active arms, designed to evaluate the effectiveness of AOT based on pathological model (PAM-AOT) as compared to a standard AOT based on TDM (TDM-AOT).
SETTING: The 3-week AOT program was administered in a clinical setting. For some patients, the treatment was delivered at participant's home with the remote support of the physiotherapist (tele-rehabilitation).
POPULATION: Twenty-six children with UCP (mean age 10.5±3.09 years; 14 females) participated in the study, with the experimental group observing a pathological model and the control group observing a typically developed model.
METHODS: Motor assessments included unimanual and bimanual ability measures conducted at T0 (baseline, before the treatment), T1 (3 weeks after T0), T2 (8-12 weeks after treatment) and T3 (24-28 weeks after treatment); a subset of 16 patients also underwent fMRI motor assessment. Generalized Estimating Equations models were used for statistical analysis.
RESULTS: Both groups showed significant improvement in bimanual function (GEE, Wald 106.16; P<0.001) at T1 (P<0.001), T2 (P<0.001), and T3 (P<0.001). Noteworthy, the experimental group showed greater improvement than the control group immediately after treatment (P<0.013). Both groups exhibited similar improvement in unimanual ability (GEE, Wald 25.49; P<0.001). The fMRI assessments revealed increased activation of ventral premotor cortex after treatment in the experimental compared with control group (GEE, Wald 6.26; P<0.012).
CONCLUSIONS: Overall, this study highlights the effectiveness of PAM-AOT in achieving short-term improvement of upper limb ability in children with UCP.
CLINICAL REHABILITATION IMPACT: These findings have significant implications for rehabilitative interventions based on AOT in hemiplegic children, by proposing a non-traditional approach focused on the most functional improvement achievable by imitating a pathological model.

(*Cite this article as:* Errante A, Beccani L, Verzelloni J, Maggi I, Filippi M, Bressi B, *et al*. Effectiveness of action observation treatment based on pathological model in hemiplegic children: a randomized-controlled trial. Eur J Phys Rehabil Med 2024;60:643-55. DOI: 10.23736/S1973-9087.24.08413-2)

KEY WORDS: Cerebral palsy; Mirror neurons; Rehabilitation; Upper extremity.

In the last decades, several interventions have been introduced to promote the improvement of upper limb abilities in children with unilateral cerebral palsy (UCP),[1] such as injections of botulinum toxin-A (BoNT-A), constraint-induced movement therapy (CIMT), and hand-arm intensive bimanual training (HABIT). Most of these models of rehabilitative intervention are based on motor skill learning theories (*e.g.*, intensive activity-based, goal-directed, and bimanual interventions).

Another effective rehabilitative approach consists in the systematic observation of actions performed by another individual, followed by their immediate reproduction (action observation treatment [AOT]).[2] This type of intervention induces an automatic activation of the motor system, which in turn promotes motor learning mechanisms, and can be easily transferred to a home-based program.[3, 4]

AOT is based on the properties of the mirror neuron system (MNS), which activates both during observation and execution of goal-directed motor acts. This system includes the inferior parietal lobule (IPL) and the ventral premotor cortex (PMv), plus the caudal part of the inferior frontal gyrus (IFG).[5-7] It has been demonstrated that the MNS is already present in typically developed children[8, 9] as well as in patients with UCP.[10] These findings support the use of AOT as a successful tool for the recovery of upper limb in children. The first pilot study suggesting the positive effect of AOT in developmental rehabilitation was conducted in children with CP.[11] Subsequently, the effectiveness of AOT was demonstrated also in a randomized controlled trial (RCT) on a cohort of UCP children.[12] In the last ten years, several studies confirmed that AOT is a promising intervention for upper limb rehabilitation in UCP,[13-15] also when combined with other treatment approach as CIMT,[16] and can also be easily transferred to a home-based program.[4, 17]

The standard version of AOT consists in the observation followed by imitation of a typically developed individual, which is proposed as model (TDM). However, an important assumption is that the MNS is activated stronger when the visual description of the observed action is matched with its corresponding motor representation in the observer's brain (direct matching hypothesis).[18] Indeed, several studies[19, 20] showed that observed actions belonging to the motor repertoire of the observer are matched with her/his motor system, while those belonging only to her/his visual experience are processed based on visual features, without involving any activation of the motor system. Thus, given the restricted motor repertoire of UCP children, they might have a higher motor resonance during observation of actions performed by a pathological hand model, more similar to their own hand, than during observation of hand actions performed by a TDM, who is completely outside their motor potential. In line with this hypothesis, a recent fMRI study[21] demonstrated that the activation of the MNS in UCP children is stronger during the observation of goal-directed actions performed by a pathological ameliorative model (PAM), as compared with a TDM. PAM consists in a paretic upper limb having a motor repertoire comparable to that of the observer (*i.e.*, a similar kinematic manipulation pattern), but ameliorative in terms of performance level.

Based on this neural evidence, the main aim of the present RCT is to verify the superiority of AOT based on PAM (PAM-AOT), with respect to standard AOT based on TDM (TDM-AOT), in the functional rehabilitation of upper limb of patients with UCP. To this aim, clinical effects at short-term, medium-term, and long-term after intervention were assessed, evaluating, as main outcome, the bimanual hand function and, as secondary outcomes, the quality of unilateral upper limb movements, manual ability and performance in activities of daily living. A second aim of the study is to measure the brain functional changes after the application of the PAM-AOT and the TDM-AOT interventions and to assess a possible difference between brain activations produced by the two treatments.

## Materials and methods

### Study design

This study consists in a prospective randomized controlled, evaluator-blinded trial, with two active arms, designed to evaluate the effectiveness of AOT based on pathological model (PAM-AOT) as compared to a standard AOT based on TDM (TDM-AOT). The extended version of the protocol has been fully described elsewhere[22] and will be briefly summarized here. The study was conducted in the Unit for Children Rehabilitation of the Santa Maria Nuova Hospital (UDGEE, AUSL-IRCCS of Reggio Emilia, Italy). The trial was approved by the Area Vasta Emilia Nord Ethics Committee (AVEN; Protocol N. 133117, November 29, 2018) and it was prospectively registered on ClinicalTrials.gov (Identifier: NCT04088994). All the recruited children agreed to participate, and their parents or caregivers provided written informed consent. The details of the study design are reported according to CONSORT guidelines (Figure 1).

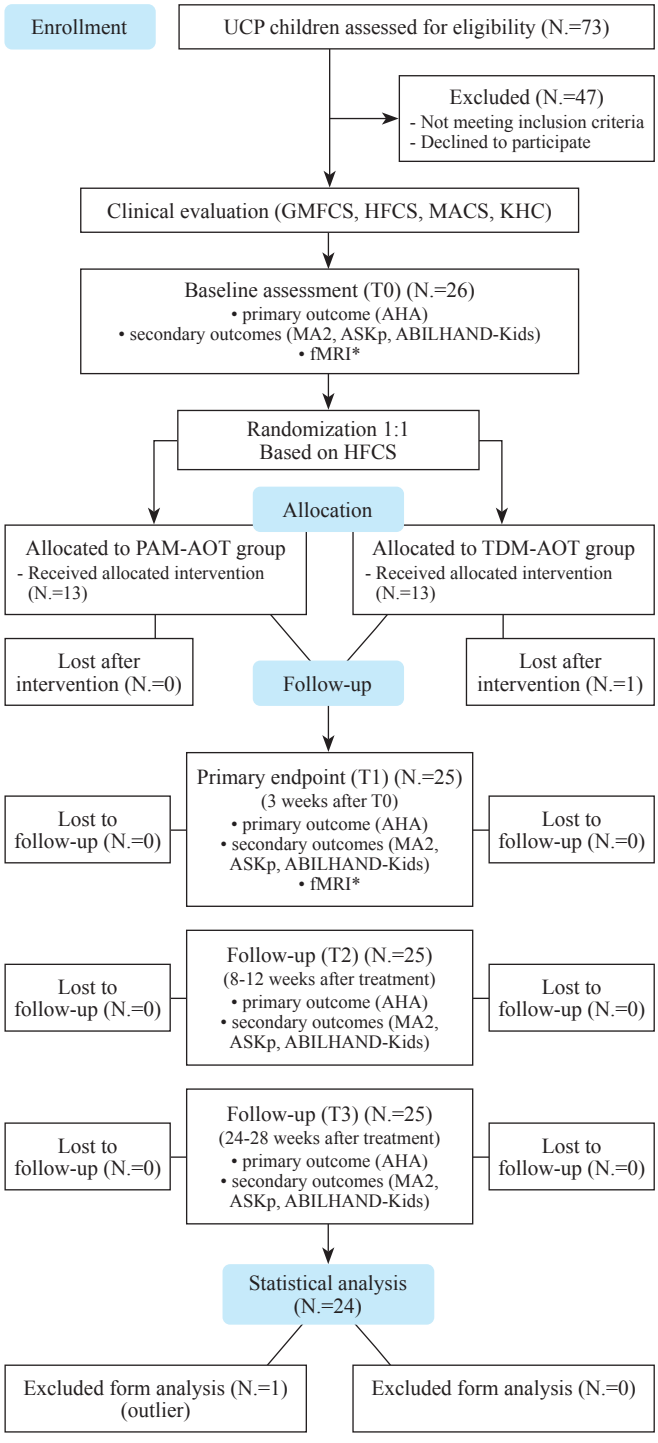


Figure 1.—Flow chart of RCT study according to CONSORT guidelines. AHA: Assisting Hand Assessment; ASKp: Activities Scale for Kids, performance version; HFCS: House Functional Classification System; MA2: Melbourne Assessment 2; PAM: pathological ameliorative model; TDM: typically developed model.
*Carried out only in a subgroup of 16 patients.

### Participants and randomization

Children with UCP were screened for eligibility from September 2019 to September 2022. The inclusion criteria were: 1) age 6-16 years at the time of recruitment; 2) confirmed diagnosis of UCP according to the definition of CP,[23] supported by MRI and clinical history; 3) predominant spasticity rather than dystonia or weakness interfering with upper limb function, according to the definition of motor type;[24] 4) mild or moderately severe functional impairment of paretic hand, from level 4 (poor active assist) to level 7 (partial spontaneous use), according to the House Functional Classification System (HFCS);[25] 5) levels of Manual Ability Classification System (MACS)[26] ranging from I to III, (f) sufficiently cooperative to participate in AOT. Exclusion criteria were: 1) presence of sensory impairment and/or uncorrected visual impairment of central origin; 2) severe cognitive disability, controlled by administration of Raven's Colored Progressive Matrices (equivalent score ≥2);[27] 3) drug-resistant epilepsy; 4) previous orthopedic surgery on the upper limb within 8 months prior to study recruitment; 5) BoNT-A injection at the upper limb within 6 months prior to study recruitment.

### Interventions

In PAM-AOT treatment, patients watched video sequences of goal-directed actions, and then reproduced them using the same objects. Videorecorded actions were performed by a pathological model (see "Set up and materials for the intervention" in Supplementary Digital Material 1: Supplementary Text File 1). In TDM-AOT patients watched video sequences of goal-directed actions performed by a typically developed model and then reproduced them. Therefore, both groups were exposed to active AOT interventions, the only difference being the type of observed model.

The duration of daily sessions was about 1 hour per day. The frequency of the intervention was 3 days per week, for the first 2 weeks, and 2 days for the last week (as a whole, 8 hours of treatment). Both treatments included both unimanual and bimanual exercises of increasing complexity. Each exercise required a maximum of 20-25 minutes to be completed. Thus, in each session they performed two exercises. The first eight exercises consisted in unimanual actions performed by the more affected hand, while the remaining seven were bimanual actions, in which the more affected hand was used as support. For the complete list of the specific exercises proposed to the patients according with the different HFCS level, see Supplementary Digital Material 2 (Supplementary Table I). During the treat-

ment, participants observed each action for a maximum of 3 minutes (range of video duration: 2.5-3 minutes) and then were requested to perform the observed action at least 3 times, within a period of maximum 3 minutes, using the same objects and setting shown in the video.

For 10 patients (N.=5 assigned to PAM-AOT and N.=5 to TDM-AOT), the treatment was delivered at participant's home with the remote support of the physiotherapist (tele-rehabilitation).

### Assessment

Clinical evaluation was performed at T0 (baseline, within 72 hours before the treatment), T1 (3 weeks after T0), T2 (8-12 weeks after treatment) and T3 (24-28 weeks after treatment) (Figure 1).

### Outcome measures

The primary outcome to assess the superiority of the pathological model (PAM) over the TDM was the change in spontaneous use of the paretic hand from baseline (T0) to follow-up (T1-T3). This outcome was measured using the AHA (V.4.4),[28] which is a performance-based test developed for children with UCP. The AHA is administered through a semi-structured play session, which requires bimanual handling and lasts 15 min. The scale uses a Rasch measurement model, which is a method to convert raw scores into a linear measure (0-100 AHA units), used for the statistical analysis. The smallest detectable difference (SDD) indicates that a change in AHA scores of 5 units or more[29, 30] can be considered a significant clinical improvement with 95% probability. The inter-rater reliability of the AHA in children with UCP is very high, with an intraclass correlation coefficient (ICC) of 0.97. The intra-rater reliability is also high, with ICC values ranging from 0.91 to 0.99.[28] Secondary outcome measures included:

- quality of the unilateral upper limb movements, measured by the Melbourne Assessment 2 (MA2).[31] MA2 measures four elements of UL movement quality, *i.e.*, movement range, accuracy, dexterity, and fluency. It comprises 14 test items of reaching, grasping, releasing, and manipulating simple objects. A raw score is provided for each of the four elements, analyzed separately. Randall *et al.*[32] reported excellent inter-rater reliability of MA2, with an ICC ranging from 0.86 to 0.98 for different subscales of the assessment. The intra-rater reliability is also high, with ICC values ranging from 0.89 to 0.98;
- performance of activities of daily living, measured by the Italian version of the Activities Scale for Kids, performance version (ASKp);[33] the ASKp is a measure of physical disability designed for children with impairments due to musculoskeletal disorders, which has also been validated in children with CP.[33, 34] The questionnaire focuses on the child's performance of activities that most often are executed at home, at school and on the playground. This scale indicates the children's perspectives of their disability and provides the option of examining performance. The ASKp showed excellent inter-rater reliability, with ICC of 0.99,[35] and good internal consistency in the Italian version, with Cronbach's α of 0.91 (CIT);[33]
- manual ability, measured by the ABILHAND-Kids,[36] a semi-structured questionnaire on a three-point ordinal scale (impossible, difficult, and easy) that measures daily manual activities referred to the activity domain of International Classification of Functioning, Disability and Health (ICF).

Parents were instructed to rate their child's perceived difficulty in performing each activity, without technical or human assistance, regardless of the limb(s) and the strategies used. The questionnaire has been developed using a Rasch measurement model, which provides a method to convert the ordinal raw scores into a linear logit measure located on a unidimensional scale, used for statistical analysis. Arnould *et al.*[36] reported ICC values ranging from 0.85 to 0.98 for the test-retest reliability of the ABILHAND-Kids scale in children with CP.

### Blinding and allocation concealment

Participants were stratified according to the HFCS (levels 4-5 *versus* level 6-7). Sequence generation was performed by random number generator, with a 1:1 allocation ratio. Allocation was concealed by central randomization, which was performed by an independent physiotherapist (S.C.) who had no clinical involvement in the trial. In particular, permuted blocks of random sizes have been used. No other data besides the initials of the name, the day of enrollment, and the HFCS level were known to this physiotherapist. Participants and their caregivers were informed about the study aims and procedures, but they were blinded to group allocation. Clinical assessment was performed by physiotherapists blinded to group allocation (M.F., I.M.). All physiotherapists involved in the application of treatments (L.B., J.V., B.B.) were not involved in clinical assessment.

### Statistical analysis

According to Consolidated Standards of Reporting Trials guidelines,[37] the estimated sample size was based on projected treatment effects on the primary outcome measure

(AHA) score.[28] The responsiveness of this scale to change induced by treatments has been estimated by Eliasson *et al.*[38] who reported an effect size of 1.16 in a population of children with UCP. Power analysis (software nQueryAdvisor) indicated that, to detect an effect size of 1.16, with a significant level of P<0.05 and statistical power of 80%, a minimum sample of 13 participants per group is required.

Clinical data were analyzed using IBM SPSS Statistics, version 28.0 (IBM Corp., Armonk, NY, USA). Demographic and clinical data were compared using independent sample *t*-test, Pearson Chi-squared Test, or Fisher's Exact Test. These comparisons were performed after the normality of variable distributions had been evaluated using the Shapiro-Wilk Test. Means and standard error of the primary and secondary outcome measures scores at each time point (T0, T1, T2 and T3) were calculated. As first step, differences between groups for all the outcome measures were investigated at baseline (T0) by means of independent sample *t*-test. Then, the primary and secondary outcomes were analysed using a Generalised Estimating Equations (GEE) model characterised by *treatment* as between-subject factor and *time* (T0, T1, T2 and T3) as within-subjects factor. Moreover, *time x treatment* interaction was also calculated. This methodology takes into account the autocorrelation intrinsic in the nature of repeated measures over time. If the effect identified using GEE analysis was statistically significant (Wald Test), a pairwise comparison was performed using the estimated marginal means to identify the two measurement points at which a significant difference occurred. The P value was adjusted based on Sidak correction for multiple comparisons in *post hoc* analysis. All statistical analyses were considered significant at P<0.05.

*Brain imaging assessment*

MR images were acquired with a 3T General Electric scanner (MR750 Discovery) equipped with a 32-channel receiver head-coil. Technical parameters of the employed sequences are reported in Supplementary Text File 1. The fMRI assessment was carried out in two sessions, one before (T0, within 72 hours) and one immediately after the end of the intervention (T1, within 72 hours), on a subgroup of patients equally distributed between the experimental and the control group. Additional criteria to participate in the fMRI study were the following: 1) compliance to participate to neuroimaging studies lasting approximately 45 minutes; 2) absence of specific contraindications to perform MRI investigations.

During the fMRI sessions participants performed a motor task in which they had to execute grasping actions (EXE GRASP) or simple movements (EXE MOVE), using their paretic hand. In each trial, the patient started with the paretic hand open in a starting posture. In EXE GRASP, the experimenter, present inside the MR room, gave one of three objects (sphere, cylinder, cube) to the patient, according to auditory instruction provided by headphones. In EXE MOVE, the patient performed opening/closing movements with the paretic hand. Visual instructions were presented by means of a digital goggles system (Resonance Technology, Northridge, CA, USA) (see Supplementary Text File 1 for a full description of the fMRI task procedure).

Data processing was performed with SPM12 (Wellcome Department of Imaging Neuroscience, University College, London, UK; http://www.fil.ion.ucl.ac.uk/spm) running on MATLAB R2021 (The Mathworks, Inc.). In order to combine data from UCP children in a group analysis, we designated the left hemisphere as the ipsilesional hemisphere (IL), contralateral to the paretic hand, and the right hemisphere as the contralesional one (CL), thus contralateral to the less-affected hand. A complete description of preprocessing pipeline is described in Supplementary Text File 1. Statistical analysis was performed using a random-effects model,[39] implemented in a two-level procedure.

At second-level, a group-based statistical analysis was performed. The t-contrast images corresponding to EXE GRASP and EXE MOVE calculated in the first-level models (T0, T1 sessions) were entered in a flexible ANOVA with sphericity-correction for repeated measures. The model included four regressors (T0 EXE GRASP, T0 EXE MOVE, T1 EXE GRASP, T1 EXE MOVE). At this level, the activations related to each condition measured at T1 *versus* T0 were contrasted in order to assess the functional reorganization associated with the rehabilitation treatments.

In order to investigate possible differential effects to the application of the PAM-AOT *versus* TDM-AOT intervention, a region of interest (ROI) analysis was performed in the areas resulted more activated after the treatment (T1 *versus* T0 contrasts) in the two main conditions (EXE GRASP, EXE MOVE). The procedure for ROI localization and BOLD signal extraction is described in Supplementary Text File 1. Between-group differences in BOLD activation was evaluated at T0, by means of Mann-Whitney U independent sample test, to verify that the two groups had the same baseline. Then, we used a GEE model characterized by *treatment* as between-subject factor and *time* (T0, T1) as within-subjects factor. In particular the interaction

was time x treatment was tested. To verify the presence of a possible linear relation between BOLD change during EXE GRASP/EXE MOVE and the motor improvement assessed with the primary outcome measure (AHA) and the other secondary outcomes, linear regression analyses were performed separately for each ROI defined using the group analysis.

## Results

### Recruitment and study sample

A total of 26 children were enrolled, out of 73 assessed for eligibility. The remaining 47 children were excluded because either they did not meet the inclusion criteria or caregivers declined to participate. Eligible patients were allocated to the PAM-AOT group (N.=13) and the TDM-AOT group (N.=13) (Figure 1). The full list of the recruited patients, with demographic and clinical information, is reported in Supplementary Digital Material 3 (Supplementary Table II). Demographic information and clinical description about the patients allocated in the experimental and control groups are reported in Supplementary Digital Material 4 (Supplementary Table III). They were matched for age, sex, more affected side, type of kinematic manipulation pattern (KHC), MACS level and HFCS levels (Supplementary Table III). See also Supplementary Digital Material 5 (Supplementary Table IV) for a description of kinematic and functional features of synergic hand and semi-functional hand according with KHC. All patients

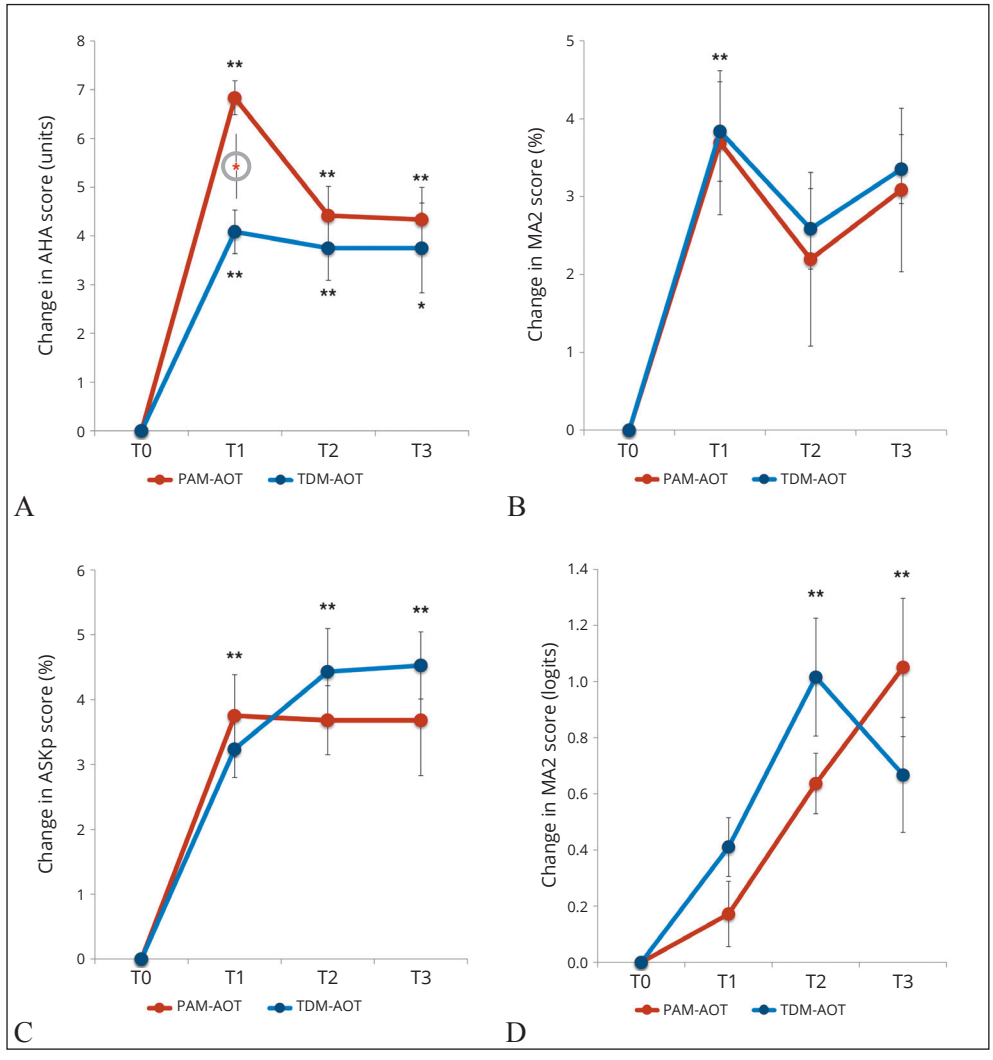


Figure 2.—Mean change over time in the primary and secondary outcome measures. Score changes in AHA (A), MA2 (B), ASKp (C) and ABILHAND-Kids (D) are reported separately for PAM-AOT group (red line in the online version) and TDM-AOT group (blue line), at T1 (3 weeks after T0), T2 (8-12 weeks after treatment) and T3 (24-28 weeks after treatment). Error bars represent mean standard error (SEM). Black asterisks indicate statistically significant effects of *time* for each measure. The circled asterisk on AHA indicates significant time-by-treatment effect on AHA.

completed the assigned intervention program (100% compliance), and no adverse event was detected. However, one patient allocated to the control TDM-AOT group was lost at the first follow-up, due to SARS-COVID infection. Another patient allocated to the PAM-AOT group was subsequently identified as outlier due to the distance in scores with respect to the sample, and excluded from the analysis to improve the statistical power. Therefore, the sample included in the statistical analysis consisted of 24 UCP patients.

Supplementary Table III shows that the experimental and control groups had similar demographic and clinical baseline characteristics. Moreover, no differences in baseline scores for the AHA ($t_{1,22}$=0.388; P=0.702), MA2 ($t_{1,22}$=0.777; P=0.446), ASKp ($t_{1,22}$=0.821; P=0.420), and ABILHAND-Kids ($t_{1,22}$=0.709; P=0.486) between groups were found. Figure 2 and Supplementary Digital Material 6 (Supplementary Table V) show the within-group change over time and between-group results for the primary and secondary outcome measures. These results are presented in the following sections, separately for the two types of outcome measures.

## Primary outcome

### *Assisting hand assessment*

The GEE model revealed a significant improvement over *time* at the primary outcome (GEE, Wald 106.16; P<0.001) (Figure 2A) (Supplementary Table V). Both PAM-AOT and TDM-AOT groups showed a significant improvement immediately after treatment (T1; P<0.001) and at follow-up (T2, P<0.001; T3, P<0.001) compared with T0 (Supplementary Table II). In addition, a significant time-by-treatment interaction was also present (GEE, Wald 8.69; P<0.034). Between-group difference in the AHA change was statistically significant, in particular immediately after treatment (T1, P=0.013), while at follow-up no significant between-group differences were present (T2, P=0.703; T3, P=0.791). The mean change in the PAM-AOT group at T1 (mean change 6.83 units, SE=0.73) was greater than the recommended SDD of 5 units, while in the TDM-AOT it was at the limit of the clinical significance (mean change 4.50 units, SE=1.09).

## Secondary outcomes

### *Melbourne assessment 2*

Both groups showed very similar improvement over *time* at MA2 (GEE, Wald 25.49; P<0.001) (Figure 2B) (Supplementary Table V). *Post-hoc* comparisons showed a significant improvement immediately after treatment (T1; P<0.001), but not at follow-up (T2, P=0.199; T3, P<0.071). No significant interaction time-by-treatment was evident (GEE, Wald 0.03; P=0.998), indicating a similar improvement in both groups. Concerning MA2 subscores (Supplementary Digital Material 7: Supplementary Table VI), a significant effect of *time* was present considering range of movement (ROM) (GEE, Wald 8.04; P=0.045) and Dexterity (GEE, Wald 14.15; P=0.003). In both subscores, *post-hoc* comparisons showed a significant effect only at T1 compared to T0 (statistical details of significant and not-significant effects related to the four MA2 subscores are reported in Supplementary Table VI).

### *Activities Scale for Kids - performance*

A similar improvement over Time at ASK-p was present in both groups (GEE, Wald 33.18; P<0.001) (Figure 2C) (Supplementary Table V). *Post-hoc* analysis showed a significant change immediately after treatment (T1; P<0.001) and at both follow-up (T2, P<0.001; T3, P<0.001) compared with baseline (Supplementary Table V). The interaction time-by-treatment was not significant (GEE, Wald 0.84; P=0.838).

### *ABILHAND-Kids*

Both groups showed significant improvement over *time* at ABILHAND-Kids (GEE, Wald 33.18; P<0.001) (Figure 2D) (Supplementary Table V). In particular, *post-hoc* comparisons showed a significant gain at both follow-up (T2, P<0.001; T3, P=0.006), but not immediately after treatment (T1; P=0.056). The interaction time-by-treatment was not significant (GEE, Wald 4.13; P=0.248).

### *Brain imaging results*

The PAM-AOT and TDM-AOT treatments, taken together, determine higher activations in several cortical and subcortical areas (Figure 3) (Supplementary Digital Material 8: Supplementary Table VII). In particular, during the execution of grasping acts (EXE GRASP), higher cortical activations after treatment (T1 *versus* T0) were present in ventral premotor cortex (PMv) and anterior middle cingulate cortex (aMCC) of the ipsilesional hemisphere. In addition, enhanced activations were found also in the posterior sector of middle cingulate cortex (pMCC) and insula of the contralesional hemisphere. Interestingly, higher activations were also present in the sensorimotor

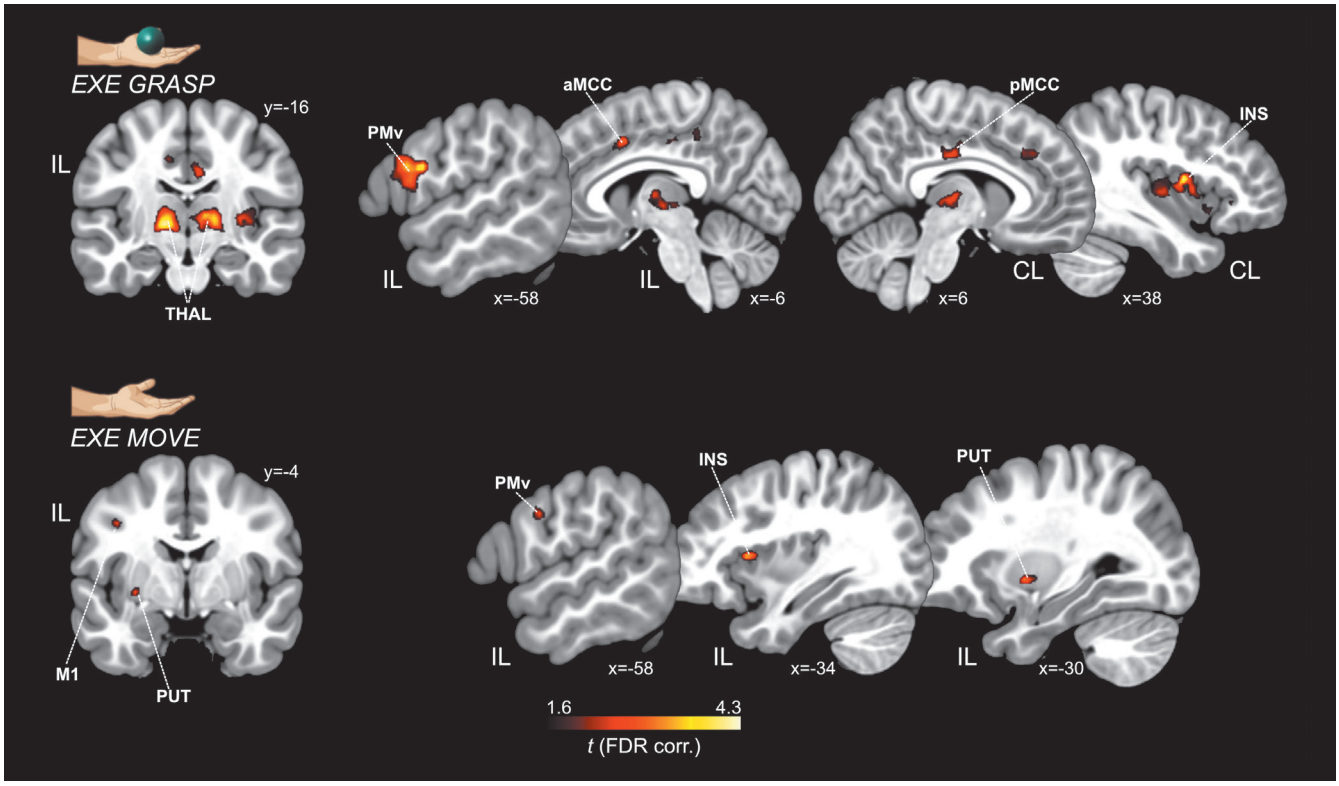


Figure 3.—Clusters of activations after the application of the rehabilitative treatments. Activation maps during the execution of grasping actions and simple movements are overlaid into a standard MNI template (ch2better, MRIcron) and shown in representative coronal and sagittal sections. The maps were calculated considering altogether the common activation change in both experimental and control groups.
aMCC: anterior sector of middle cingulate cortex; CL: contralesional; IL: ipsilesional; INS: insula; M1: primary motor cortex; PMv: ventral premotor cortex; pMCC: posterior sector of middle cingulate cortex; PUT: putamen; THAL: thalamus.

thalamus, bilaterally. Concerning the execution of simple movements (EXE MOVE), the activations at T0 and T1 were comparable, even if some small clusters of enhanced activations were present in the ipsilesional primary motor area (M1), PMv, Putamen, and dorso-central insula.

Concerning EXE GRASP condition, 3 ipsilesional (IL) ROIs were defined: PMv (x=-58, y=-2, z=34), aMCC (x=-4, y=12, z=40) and Thalamus (x=-12, y=-18, z=2). Supplementary Digital Material 9 (Supplementary Figure 1) shows the individual position of PMv and Thalamus ROIs. Other 3 contralesional (CL) ROIs included: Insula (x=40, y=0, z=8), Thalamus (x=16, y=-16, z=4) and pMCC (x=6, y=-18, z=38). No between-group differences in activation at T0 were found in the considered ROIs (Figure 4) (IL PMv, P=0.74; IL aMCC, P=0.66; IL Thal, P=0.45; CL Ins, P=0.24; CL pMCC, P=0.36; CL Thal, P=0.45). However, concerning the IL PMv, the GEE model showed a significant interaction time-by-treatment (GEE, Wald 6.26; P<0.012). *Post-hoc* comparison indicated that following the application of the intervention (T1), the PAM-AOT group had higher activation of PMv as compared to the TDM-AOT group (P<0.05). No other significant interaction time-by-treatment at T1>T0 were present in the remaining ROIs. Concerning *EXE MOVE* condition, 4 ROIs in the ipsilesional hemisphere were included in the analysis: M1 (x=-40, y=-4, z=38), PMv (x=-60, y=0, z=34), Putamen (x=-30, y=0, z=2), and Insula (x=-42, y=-6, z=6). No between-group differences in activation at T0 were found in the considered ROIs (P>0.05). Moreover, no significant interaction time-by-treatment were statistically significant.

The regression analysis revealed a positive linear relation between change at T1>T0 in BOLD activation during EXE GRASP in ipsilesional PMv and change of AHA score ($R^2$=0.49; P=0.04) (Figure 4B). A similar linear relation was also present at subcortical level, between the BOLD change in ipsilesional Thalamus and change in AHA score ($R^2$=0.78; P<0.001) (Figure 4C). During EXE MOVE, no significant relation between change at T1>T0 in BOLD ac-

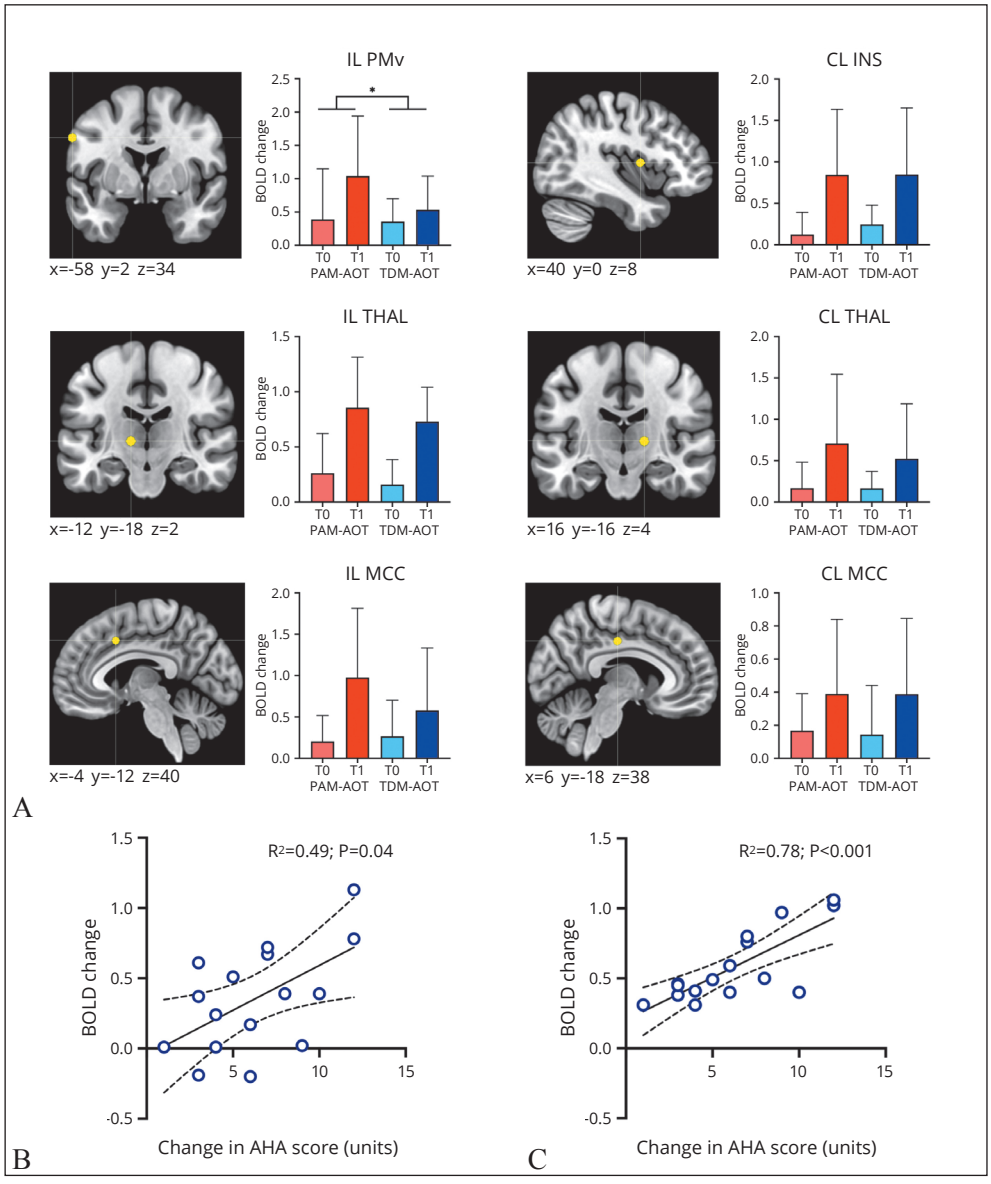


Figure 4.—Results of ROI analysis: A) the histograms show the averaged magnitude of activation (% BOLD signal change) measured at T1, and compared with T0, in each ROI. For each histogram, on the left side, the position of each corresponding ROI is presented as yellow colored sphere. Vertical lines in the histograms indicate standard error mean (SEM). Asterisk indicates significant interaction time-by-treatment (GEE, Wald 6.26; P<0.012) in ventral premotor cortex. B, C) Scatterplots show the linear relation between BOLD change in the ipsilesional ventral premotor cortex and thalamus, respectively, at T1 *vs*. T0, and clinical change assessed with the primary outcome measure (AHA). Dashed lines indicate 95% confidence intervals.
CL: contralesional; IL: ipsilesional; INS: insula; MCC: middle cingulate cortex; PMv: ventral premotor cortex; THAL: thalamus.

tivation and improvement in AHA score was found, however a significant linear relation was present with improvement in MA2 score in ipsilesional M1 ($R^2$=0.53; P<0.001), and Putamen ($R^2$=0.47; P<0.003) (Supplementary Digital Material 10: Supplementary Figure 2).

## Discussion

This RCT study aimed at demonstrating the effectiveness of the AOT based on PAM, and its superiority, as compared to standard AOT based on TDM, for the improvement of upper limb ability of children with UCP. To the best of our knowledge, this is the first RCT study ever done in UCP children demonstrating the benefit of the use of a pathological model within AOT treatment.

### Differential improvements in outcome measures immediately after AOT and at follow-up

In general, AOT, originally applied to rehabilitation of adult patients suffering mainly from stroke and Parkinson's disease[2, 40] has been proven to be also effective on

UCP patients.[11, 12] The positive effects are observed especially in the use of the more affected hand in bimanual tasks (assessed with the AHA). The present work confirms these effects in UCP children of both groups. However, only patients treated with PAM-AOT showed a clinically relevant improvement (change of about 7 AHA units, higher than SDD), while the improvement of the TDM-AOT group was lower (about 4.5 AHA units) than the SDD. Note that this latter effect is in line with those reported in similar RCT studies on UCP based on a typically developed model.[12, 14]

Besides the effect observed in bimanual activities, both groups showed comparable statistical improvement, after treatment, in the unimanual performance of the more affected hand, as assessed with MA2. This finding is in line with previous data,[12] suggesting that action observation elicits a marked propensity to preserve the task proficiency, by selecting movements that guarantee the achievement of the action goals, independently from the kinematic resemblance with the observed model. The highest effect observed in AHA with respect to MA2 is in agreement with the functional goal of the AOT, which does not consist in improving the isolated use of the more affected hand, but in promoting its active use in bimanual tasks.

The results of ASKp at T1 show a significant improvement in both groups, but without difference between them, and ABILHAND-Kids scale did not show any effect. This latter result could be due to the high score observed at baseline in both groups. The results on the two scales could be due to the fact that they evaluate aspects different from AHA, such as manual daily activities, independently of whether the patient uses the more affected or the less affected hand, or both.

Follow-up scores show that in both groups the AHA changes were maintained at T2 and T3. The difference between groups was significant only at T1, very likely due to a slight decrease of the effect in the PAM-AOT group at T2 and T3. A possible explanation could be that children of PAM-AOT group, after the end of treatment, came back to interact in daily life with healthy individuals, who show kinematic models quite different from their own. Conversely, patients of the TDM group had greater continuity between the model observed during AOT training and the healthy kinematic models of daily life. If this is true, additional PAM-AOT sessions in compliant children could strengthen the effectiveness of the treatment.

Concerning MA2, the changes were not maintained at follow-up, both in terms of overall score level and in the ROM and Dexterity subscores. Concerning ASKp and ABILAND-Kids, both groups showed improvements at T2 and T3. This suggests a generalization of the effect of treatment to other daily life activities, which in turn should impact on patients' global functioning.

The most relevant finding of the present study is that the pathological model produced a stronger effect in terms of improved spontaneous use of the more affected hand during bimanual tasks. Our explanation is that when a UCP child observes an action performed by another individual with a similar motor repertoire, the visual description of the observed action is compared with her/his corresponding motor representation, based on the mirror matching mechanism.[20, 21] Interestingly, some patients involved in the fMRI assessment recognized the presented pathological hand model as their own hand (for a reference to a similar embodied mechanism, see Cross *et al.*).[41] On the contrary, the observation of a TDM did not produce a comparable effect, probably because the observed model did not match the motor repertoire of UCP children. This means that, although these children are able to understand the goal of the actions performed by healthy individuals, nonetheless tend to resonate stronger with models having a similar pattern of manipulation.

### Treatment-induced changes in brain activation pattern associated to clinical improvements

The clinical improvement observed in both groups was associated with significant changes in brain activation assessed during the execution, with the more affected hand, of grasping acts and simple movements. These changes were evident not only in the contralesional, but also in the ipsilesional hemisphere, suggesting possible mechanisms of adaptive plasticity also within perilesional regions. This result is also in line with previous fMRI findings in children with CP[13, 14] and adult patients with stroke,[42] showing increased activation of premotor and parietal areas following the application of AOT.

During execution of grasping acts, higher activation after AOT was evident in the ipsilesional PMv, a sector of premotor cortex involved in controlling, through direct or indirect projections, goal-directed hand movements.[43] Furthermore, PMv is a crucial node of the MNS in healthy adults[6] as well as in TD children[44] and patients with UCP.[10, 21] In agreement with the direct matching hypothesis, ROI analysis revealed that activation of PMv was greater in patients treated with PAM-AOT.

Concerning activation of ipsilesional aMCC, recent studies showed that its electrical stimulation in drug-resistant epileptic patients triggers a variety of goal-orient-

ed behaviors, including reaching and grasping actions.[45] Thalamus activation could be related to the recruitment of cortico-thalamo-cortical loops, crucial for establishing the motor routines necessary for learning new motor skills.[46] In addition, also the contralesional pMCC and mid-posterior sectors of the insula are more activated after AOT. Considering the properties of these regions,[45, 47] the greater involvement of the cingulate cortex-insula circuit could reflect the processing of proprioceptive and somatosensory information related to the grasped object.

Noteworthy, the change in activation of the ipsilesional PMv and thalamus was linearly associated with clinical improvement in bimanual control assessed with AHA, indicating that AOT may determine an increase in the functioning of cortico-subcortical circuits, improving the organization of coordinated bimanual actions.

During execution of simple movements, the increased activation, after AOT, occurred in the ipsilesional M1, central insula, and putamen. This finding is in line with previous evidence on the effects of training on motor learning in healthy people,[46] showing that improvement in motor skills is correlated to higher activation of cortico-striatal circuits.

### Feasibility aspects of AOT based on the pathological ameliorative model

Compared to previous similar trials, the present study has several advantages. First of all, we enrolled only UCP children with specific features of the more affected upper limb, using restrictive inclusion criteria. On the contrary, previous clinical trials[11, 13, 15] investigated the feasibility of AOT in different clinical forms of CP.

Concerning the duration and the frequency of the training, the type of non-intensive AOT treatment proposed here, differing from previous treatments in UCP patients,[11, 12, 48] suggests new insights about the most appropriate intensity to use in order to obtain significant clinical changes. In fact, debriefing reports from patients after the administration of the intervention confirm that the low intensity treatment makes the training more tolerable and practicable in the daily life.

Previous works on AOT carried out in children with UCP used the same set of exercises for all enrolled patients.[11, 13, 15] In the present work, the exercises consisted in unimanual or bimanual actions of increasing complexity, based on daily life activities and adapted to the age of the participants and their functional level. Another important feasibility-related aspect concerns the possibility to treat the enrolled patients at home in a tele-rehabilitation setting. This approach represents a useful option to provide AOT intervention in a user-friendly, playful rehabilitative setting for children.[4, 17, 49]

In conclusion, PAM-AOT is a new promising tool for rehabilitation of the upper limb in children with UCP. Our findings demonstrate its superiority (at least at short term), as compared to the traditional version of AOT based on TDM, thus representing a turning point for the actual rehabilitative intervention in hemiplegic children.

### Limitations of the study

A possible limitation of the present study is the small number of enrolled patients, which was, however, in accord with the power analysis carried out for the primary outcome measure. Moreover, the choice to enroll a homogeneous group of children, by means of restrictive inclusion criteria, besides a careful statistical data analysis, provides a high reliability of the obtained results.

## Conclusions

The AOT based on imitation of PAM is a new promising tool for rehabilitation of the upper limb in children with CP. Moreover, these findings demonstrate the superiority of PAM-AOT, as compared to the traditional version of AOT based on TDM, representing a turning point for the actual rehabilitative intervention in hemiplegic children. Differently from the current state of rehabilitation, in which adaptation to healthy models constitutes the objective of upper limb recovery in UCP, we propose a non-traditional logic focused on the most functional improvement attainable by imitating a pathological model, thus respecting the differences imposed by the nature, the extent and the stage of CP. A rehabilitation strategy complying with these assumptions should provide exercises calibrated to the patient's motor repertoire, in the framework of tailored medicine.

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*Conflicts of interest*
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

*Funding*
This study was funded by a grant from Chiesi Farmaceutici S.p.A. (Parma, IT). However, nobody from Chiesi Farmaceutici had a role in the design of the study or in writing this manuscript.

*Authors' contributions*
Antonino Errante and Laura Beccani contributed equally to this work. All the authors contributed to the conceptualization and design of the study. Antonino Errante have given substantial contribution to investigation, data curation, and formal analysis. Laura Beccani, Jessica Verzelloni, and Barbara Bressi carried out the rehabilitative intervention. Mariacristina Filippi and Irene Maggi acquired clinical data. Settimio Ziccarelli and Francesca Bozzetti contributed to the fMRI investigation. Stefania Costi performed allocation concealment. Leonardo Fogassi and Adriano Ferrari were responsible of funding acquisition, project administration, and supervision. Antonino Errante drafted the manuscript, and all the other authors reviewed and edited the final version. All authors read and approved the final version of the manuscript.

*Congresses*
The preliminary results of this study were presented as oral presentation at the National Congress of the Italian Society of Neuropsychology, November 18-19, 2022, Rovereto (Italy).

*Acknowledgements*
The authors would like to gratefully acknowledge the patients, the caregivers, and families for their participation in the trial.

*History*
Article first published online: May 30, 2024. - Manuscript accepted: May 2, 2024. - Manuscript revised: April 4, 2024. - Manuscript received: January 11, 2024.

*Supplementary data*
For supplementary materials, please see the HTML version of this article at www.minervamedica.it