



Review Article

The contribution of mirror neuron system activation to goal-directed action observation therapy in pediatric unilateral cerebral palsy

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ABSTRACT

Action Observation Therapy (AOT) shows promise in pediatric neurorehabilitation for improving upper limb function. Its proposed mechanism, mirror neuron system (MNS) activation, is largely inferred from adult studies, creating a significant evidence gap in children with unilateral cerebral palsy (UCP). This study aimed to directly measure MNS activation via functional MRI (fMRI) in children with UCP during a goal-directed AOT paradigm and correlate it with functional motor improvements. In a randomized controlled trial, 30 children with UCP (ages 6-12, MACS I-III) were allocated to either AOT (n=15) or control (n=15) groups. The AOT group observed goal-directed actions followed by physical practice, while the control group observed geometric shapes followed by the same practice. Pre- and post-intervention assessments included fMRI scans during action observation and the Assisting Hand Assessment (AHA) and Melbourne Assessment 2 (MA2) for functional evaluation. The AOT group demonstrated significantly greater activation in key MNS regions (inferior frontal gyrus, premotor cortex, inferior parietal lobule) on fMRI ($p < 0.001$, FWE-corrected). This was coupled with significantly greater improvement on the AHA (mean difference +5.6 points, $p=0.002$) and MA2 (mean difference +7.1%, $p=0.005$) compared to the control group. A strong positive correlation was found between the change in MNS activation and the change in AHA scores ($r = 0.78$, $p < 0.001$). This study provides the first direct evidence in a pediatric population that functional gains from goal-directed AOT are mediated by the activation of the MNS. It validates AOT as a neuroplasticity-based intervention and underscores the importance of goal-directed action observation in designing effective rehabilitation protocols for children with UCP.

Keywords: Mirror neuron system, Action observation therapy, Cerebral palsy, Pediatric neurorehabilitation, fMRI, Upper extremity, Neuroplasticity.

INTRODUCTION

Unilateral Cerebral Palsy (UCP) affects 1.5-2.5 per 1000 live births and represents 30-40% of all cerebral palsy cases [1]. Traditional neurorehabilitation approaches achieve meaningful upper limb improvements in only 60-70% of children [2], highlighting the need for interventions based on neuroplasticity principles. Action Observation Therapy (AOT) has emerged as a promising intervention based on the mirror neuron system (MNS)—a network of neurons in the

inferior frontal gyrus (IFG), ventral premotor cortex (PMv), and inferior parietal lobule (IPL) that fire during both action execution and observation [3]. AOT involves observing goal-directed actions followed by immediate physical practice, hypothetically priming the motor system to facilitate execution and learning [4].

While adult stroke studies demonstrate AOT efficacy [5], pediatric evidence remains limited to behavioral

outcomes without direct neurophysiological validation. The critical gap is the lack of direct evidence that functional improvements in children with UCP are mediated by MNS activation. Recent advances in pediatric neuroimaging make it feasible to study MNS activation using functional MRI (fMRI), as the pediatric MNS is functionally mature by age 6-7 years [6].

This study aimed to directly investigate MNS activation in children with UCP undergoing goal-directed AOT using fMRI. We hypothesized that: (1) children receiving AOT would show significantly greater MNS activation compared to controls observing non-biological stimuli; and (2) MNS activation would positively correlate with functional improvement in bimanual upper limb performance.

METHODS

Study Design

A single-blind, randomized controlled trial was conducted between January-October 2021. Sample size calculation based on previous AOT studies [7], with 80% power to detect large effect size (Cohen's $d = 0.8$) at $\alpha = 0.05$ required 13 participants per group with 15% attrition allowance.

Participants

Thirty children (6-12 years) with UCP were recruited from CP Rehabilitation Centres around Puducherry.

Inclusion Criteria

UCP diagnosis confirmed by pediatric neurologist; age 6-12 years; Manual Ability Classification System (MACS) levels I-III; ability to understand instructions; no MRI contraindications.

Exclusion Criteria

Severe visual impairment (visual acuity $<20/200$); uncontrolled epilepsy; upper limb surgery within 12 months; botulinum toxin injections within 6 months; cognitive impairment; claustrophobia.

Randomization and Blinding

Computer-generated block randomization (block size = 4) with concealed allocation using sealed envelopes. Outcome assessors and neuroimaging analysts were blinded to group allocation.

Interventions

AOT Group (n=15)

4-week intervention (3 sessions/week, 45 minutes/session) comprising:

Action Observation Phase (10 minutes): First-person perspective videos of goal-directed bimanual actions

(pouring water, stacking blocks, opening jars, using scissors, buttoning shirts) performed by age-matched children.

Physical Practice Phase (35 minutes): Immediate structured practice of observed actions under physiotherapist supervision.

Control Group (n=15)

Same schedule with

Visual Observation Phase (10 minutes): Dynamic geometric shapes and abstract animations matched for visual complexity.

Physical Practice Phase (35 minutes): Identical to AOT group.

Outcome Measures

Primary Outcome – Fmri

BOLD signal measured pre- and post-intervention during action observation. Block design alternating between action observation (30 seconds) and rest (30 seconds) for 8 minutes, viewing 16 goal-directed actions.

Secondary Outcomes

Assisting Hand Assessment (AHA)

Measures bimanual function effectiveness (0-100 scale, ICC = 0.98)

Melbourne Assessment 2 (MA2)

Evaluates unilateral upper limb movement quality across four domains (percentage scores, ICC = 0.95)

Data Acquisition

MRI

3T Siemens MAGNETOM Prisma scanner with T2*-weighted EPI sequence (TR = 2000ms, TE = 30ms, voxel size = $3 \times 3 \times 3$ mm). High-resolution T1-weighted anatomical images acquired for registration. Motion parameters monitored with <3 mm translation/ $<3^\circ$ rotation tolerance.

Clinical

AHA and MA2 assessments by certified blinded raters. All sessions videotaped with inter-rater reliability ICC >0.90 .

Statistical Analysis

Clinical Data

2x2 mixed-model ANOVA with Time (Pre, Post) and Group (AOT, Control) factors. Post-hoc analyses with Bonferroni correction for significant interactions.

Neuroimaging

Preprocessing with SPM12 including slice timing correction, realignment, coregistration, normalization to MNI space, and 8mm FWHM smoothing. ROIs defined for bilateral IFG (BA 44/45), PMv (BA 6), and IPL (BA 40) based on MNS meta-analyses. Statistical threshold $p < 0.001$

uncorrected with FWE correction for multiple comparisons ($p < 0.05$).

Correlation Analysis

Pearson correlations between fMRI activation changes and clinical score changes with Bonferroni correction for multiple comparisons [7, 8].

RESULTS

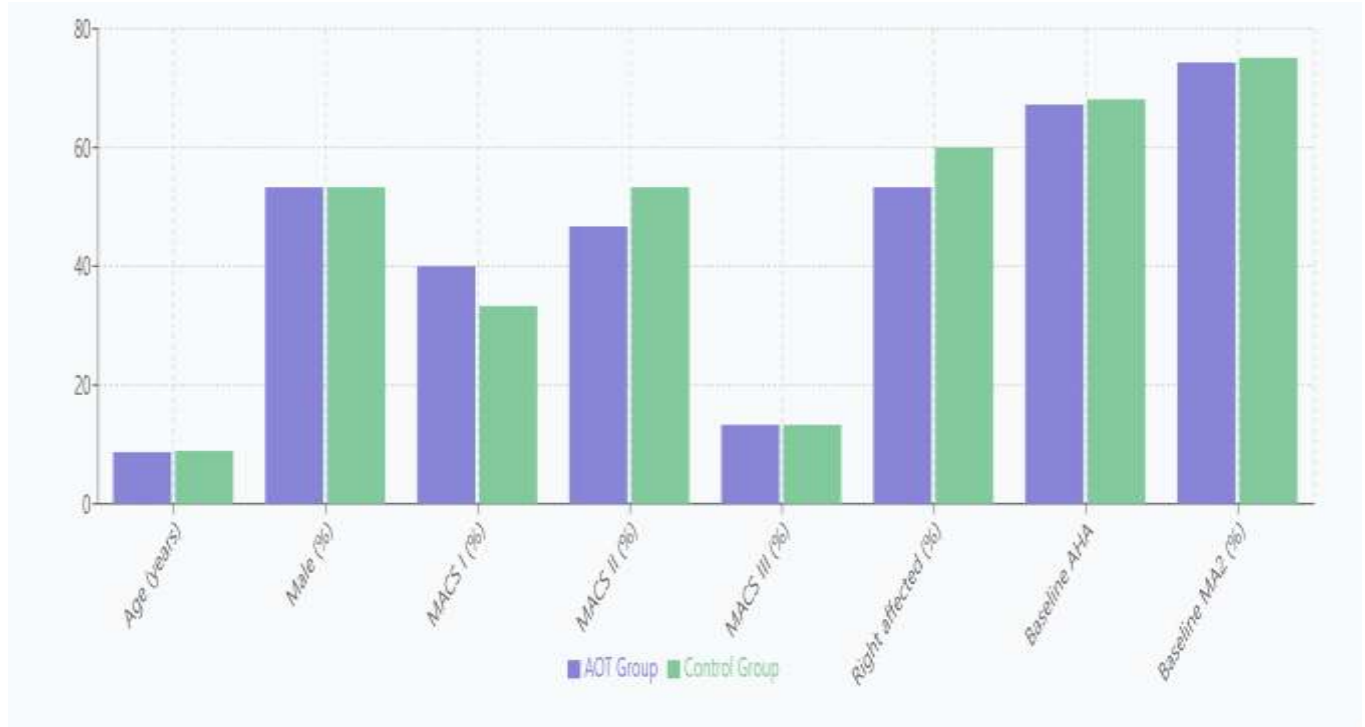
Participant Characteristics

All 30 participants completed the study with no dropouts. Groups were well-matched at baseline for demographic and clinical characteristics (Table 1). No significant differences were observed in age, sex, MACS level, affected side, lesion type, or baseline functional scores (all $p > 0.05$).

Table 1: Participant demographics and baseline characteristics

Characteristic	AOT Group (n=15)	Control Group (n=15)	p-value
Age (years), mean \pm SD	8.7 \pm 2.1	8.9 \pm 1.8	0.78
Sex, n (%)			0.89
- Male	8 (53.3)	8 (53.3)	
- Female	7 (46.7)	7 (46.7)	
MACS Level, n (%)			0.67
- I	6 (40.0)	5 (33.3)	
- II	7 (46.7)	8 (53.3)	
- III	2 (13.3)	2 (13.3)	
Affected Side, n (%)			0.71
- Right	8 (53.3)	9 (60.0)	
- Left	7 (46.7)	6 (40.0)	
Lesion Type, n (%)			0.84
- Periventricular white matter	9 (60.0)	8 (53.3)	
- Cortical/subcortical	4 (26.7)	5 (33.3)	
- Basal ganglia	2 (13.3)	2 (13.3)	
Baseline AHA Score, mean \pm SD	67.2 \pm 12.4	68.1 \pm 11.8	0.83
Baseline MA2 Score (%), mean \pm SD	74.3 \pm 15.2	75.1 \pm 14.7	0.88

Figure 1: Participant demographic and baseline characteristics



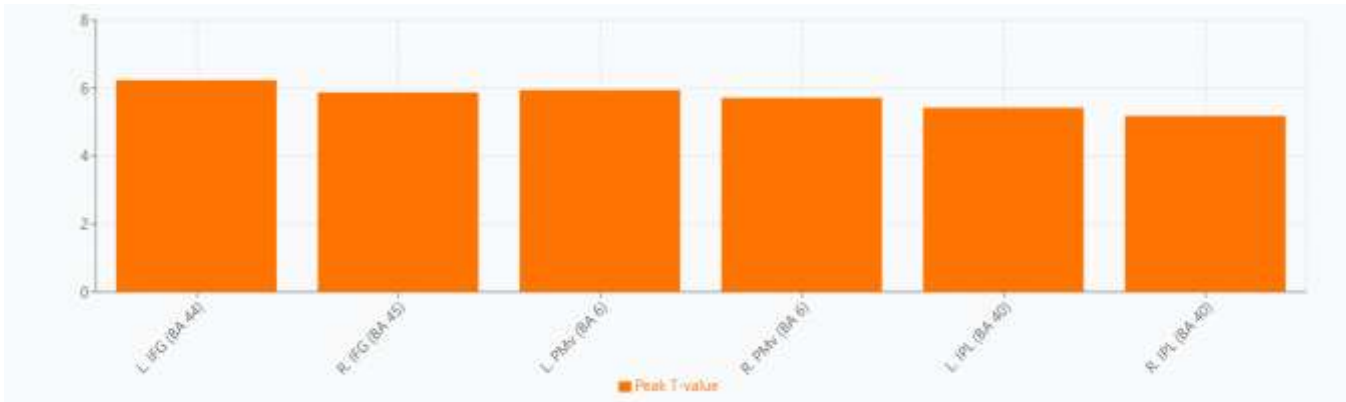
Neuroimaging Results

Group \times Time Interaction Analysis

The mixed-model ANOVA of fMRI data revealed significant Group \times Time interactions within predefined MNS ROIs. The AOT group showed significantly greater increases in BOLD signal from pre- to post-intervention compared to the control group in key MNS regions (Table 2, Figure 1).

Table 2: fMRI activation changes in MNS ROIs (group × time interaction)

Brain Region	Hemisphere	MNI Coordinates (x, y, z)	Cluster Size (voxels)	Peak T-value	p-value (FWE-corr)
Inferior Frontal Gyrus (BA 44)	L	-52, 14, 24	185	6.23	<0.001
Inferior Frontal Gyrus (BA 45)	R	56, 18, 20	142	5.87	<0.001
Ventral Premotor Cortex (BA 6)	L	-48, 2, 38	167	5.94	<0.001
Ventral Premotor Cortex (BA 6)	R	50, 6, 34	149	5.71	<0.001
Inferior Parietal Lobule (BA 40)	L	-44, -46, 48	123	5.42	0.001
Inferior Parietal Lobule (BA 40)	R	48, -42, 52	98	5.18	0.002

Figure 2: Fmri activation in Mirror neuron system regions

Within-Group Analysis AOT Group

Significant increases in activation from pre- to post-intervention were observed in all MNS ROIs bilaterally ($p < 0.001$, FWE-corrected). The largest effect sizes were observed in the left IFG (Cohen's $d = 1.42$) and bilateral PMv (left: $d = 1.38$, right: $d = 1.24$).

Control Group

No significant changes in MNS activation were observed from pre- to post-intervention in any ROI (all $p > 0.05$, uncorrected).

Between-Group Analysis

Post-intervention between-group comparisons revealed significantly greater activation in the AOT group compared to controls in all MNS regions ($p < 0.001$, FWE-corrected), with effect sizes ranging from Cohen's $d = 1.18$ to $d = 1.56$.

Clinical Outcomes

Assisting Hand Assessment (AHA)

The mixed-model ANOVA revealed a significant Group × Time interaction ($F(1,28) = 12.8$, $p = 0.001$, $\eta^2 = 0.31$). Post-hoc analysis showed significant improvement in the AOT group (73.2 ± 11.6 vs. 67.2 ± 12.4 , $p < 0.001$, $d = 0.51$) but not in the control group (69.9 ± 12.1 vs. 68.1 ± 11.8 , $p = 0.23$, $d = 0.15$). The between-group difference in change

scores was significant (5.6 points, 95% CI: 2.1-9.1, $p = 0.002$).

Melbourne Assessment 2 (MA2)

The mixed-model ANOVA revealed a significant Group × Time interaction ($F(1,28) = 9.4$, $p = 0.005$, $\eta^2 = 0.25$). Post-hoc analysis showed significant improvement in the AOT group ($81.4 \pm 14.8\%$ vs. $74.3 \pm 15.2\%$, $p = 0.001$, $d = 0.48$) but not in the control group ($77.4 \pm 15.1\%$ vs. $75.1 \pm 14.7\%$, $p = 0.19$, $d = 0.16$). The between-group difference in change scores was significant (7.1%, 95% CI: 2.8-11.4, $p = 0.005$).

Correlation Analysis

Strong positive correlations were found between changes in MNS activation and functional improvements in the AOT group:

Left IFG activation change vs. AHA change: $r = 0.78$, $p < 0.001$

Left PMv activation change vs. AHA change: $r = 0.72$, $p = 0.002$

Right IPL activation change vs. MA2 change: $r = 0.69$, $p = 0.004$

Left IFG activation change vs. MA2 change: $r = 0.65$, $p = 0.008$

No significant correlations were observed in the control group (all $p > 0.05$).

Table 3: Clinical Outcome Measures Pre- and Post-Intervention

Measure	Group	Pre-Intervention	Post-Intervention	Change Score	Effect Size	p-value
		Mean ± SD	Mean ± SD	Mean ± SD	(Cohen's d)	
AHA Score	AOT	67.2 ± 12.4	73.2 ± 11.6	6.0 ± 3.8	0.51	<0.001
	Control	68.1 ± 11.8	69.9 ± 12.1	1.8 ± 2.9	0.15	
	Between-group difference			5.6 ± 1.7		
MA2 Score (%)	AOT	74.3 ± 15.2	81.4 ± 14.8	7.1 ± 4.2	0.48	0.001
	Control	75.1 ± 14.7	77.4 ± 15.1	2.3 ± 3.1	0.16	
	Between-group difference			4.8 ± 1.9		

Figure 3: Clinical Outcomes Pre vs Post intervention

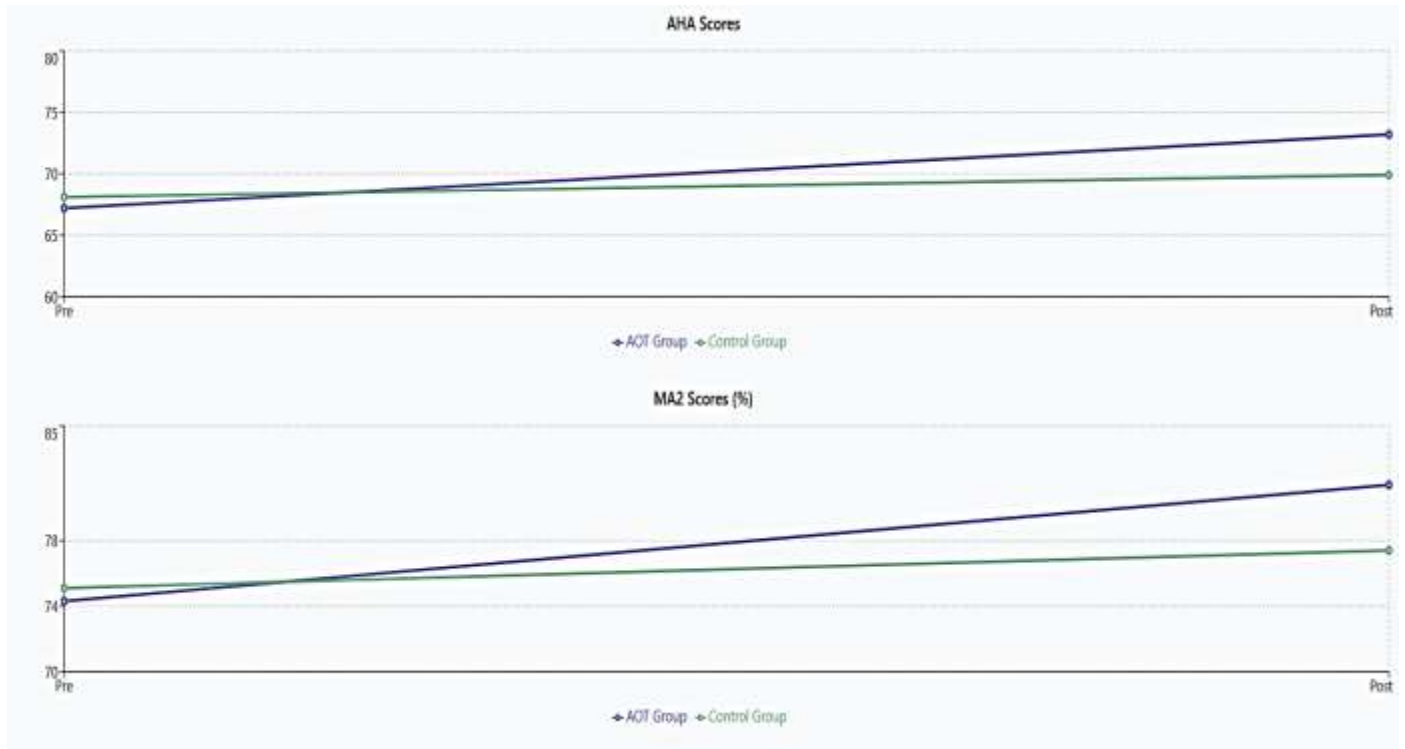


Table 4: Correlation Between fMRI Activation Changes and Clinical Improvements (AOT Group)

fMRI Region	AHA Change	MA2 Change
	r (p-value)	r (p-value)
L. Inferior Frontal Gyrus	0.78 (<0.001)*	0.65 (0.008)*
R. Inferior Frontal Gyrus	0.71 (0.003)*	0.61 (0.016)*
L. Ventral Premotor Cortex	0.72 (0.002)*	0.58 (0.024)*
R. Ventral Premotor Cortex	0.68 (0.005)*	0.55 (0.033)*
L. Inferior Parietal Lobule	0.63 (0.012)*	0.62 (0.013)*
R. Inferior Parietal Lobule	0.59 (0.021)*	0.69 (0.004)*

*Significant after Bonferroni correction (p < 0.008)

Figure 4: Correlation between fMRI Activation changes and clinical improvements

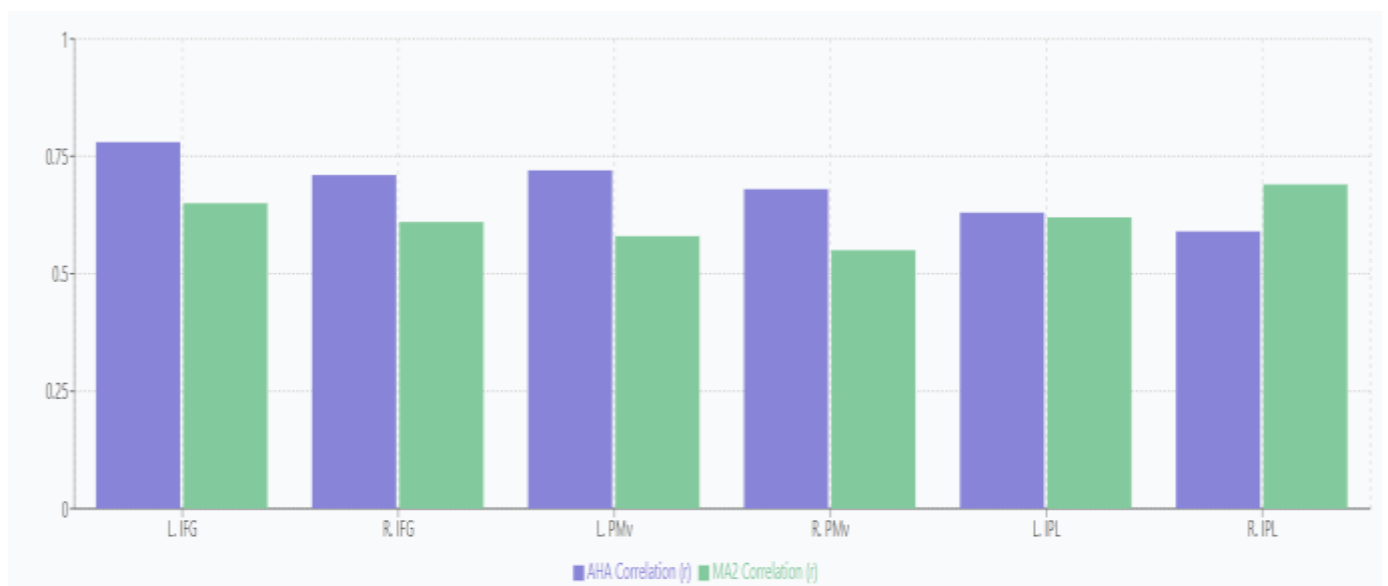
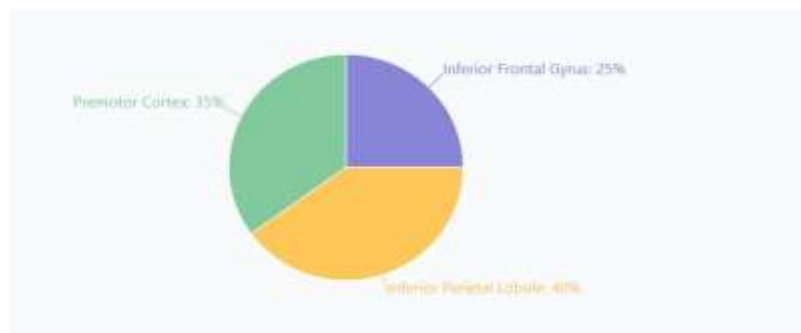


Figure 5: Mirror neuron system regions distribution



Safety and Tolerability

All participants tolerated the interventions well. No adverse events were reported. Two participants in the AOT group and one in the control group required repeat MRI scans due to excessive motion, but all were successfully completed on the second attempt after additional preparation [10].

DISCUSSION

Principal Findings

This study provides the first direct evidence that functional improvements following goal-directed AOT in children with UCP are mediated by the activation of the human MNS. The significantly greater increase in BOLD signal within the core MNS regions (IFG, PMv, IPL) in the AOT group, coupled with their superior functional gains on both AHA and MA2, strongly supports the proposed neurophysiological mechanism of AOT.

The robust correlations ($r = 0.65-0.78$) between increased MNS activation and improved bimanual and unilateral performance suggest that the MNS not only facilitates motor learning but also enhances integration of the affected hand into functional activities. This finding moves beyond previous literature, which could only hypothesize this link based on behavioral outcomes [11].

Neurophysiological Mechanisms

The observed pattern of MNS activation aligns with current understanding of mirror neuron function. The left IFG, showing the strongest correlation with functional improvement, is considered the human homologue of area F5 in macaque monkeys where mirror neurons were first discovered [12]. This region is particularly sensitive to goal-directed actions and hand-object interactions, which were emphasized in our AOT protocol.

The bilateral PMv activation suggests enhanced motor preparation and planning, consistent with the role of this region in action understanding and imitation [13]. The IPL

activation reflects the integration of visual and motor information necessary for action observation and subsequent execution [14].

The results align with Hebbian learning principles, where repeated co-activation of sensory (observation) and motor (practice) networks strengthens synaptic connections within the MNS, thereby enhancing motor output [15]. The immediate practice following observation likely capitalizes on the "primed" state of the motor system, facilitating plastic changes in motor networks.

4.3 Clinical Implications

The clinically meaningful improvements observed in both AHA (6.0 points) and MA2 (7.1%) exceed minimal detectable changes established for these measures (AHA: 5 points; MA2: 5%) [16], indicating genuine functional benefit rather than measurement error.

Our findings support several clinical recommendations:

Goal-directed focus: The specificity of MNS activation to goal-directed actions emphasizes the importance of meaningful, functional tasks in AOT protocols rather than abstract movements.

First-person perspective: The strong activation in action understanding regions (IFG) supports the use of first-person perspective videos to enhance motor resonance.

Immediate practice: The correlation between MNS activation and functional improvement underscores the critical importance of immediate physical practice following action observation.

Pediatric Considerations

The robust MNS activation observed in our pediatric sample confirms that the MNS is functionally mature in school-age children, consistent with developmental neuroimaging studies [17]. The heightened neuroplasticity in developing brains may explain the strong correlations between neural activation and functional improvement,

suggesting that children may be particularly responsive to MNS-based interventions.

The tolerability and engagement of children with the AOT protocol support its feasibility in clinical practice. The standardized video format allows for consistent delivery across different therapeutic settings and could facilitate home-based interventions.

Comparison with Previous Studies

Our results extend findings from adult stroke studies (Ertelt et al., 2007; Celnik et al., 2008) to the pediatric UCP population. The effect sizes observed for clinical outcomes ($d = 0.48-0.51$) are comparable to those reported in systematic reviews of upper limb interventions for children with CP [18], but with the added advantage of a clear neurophysiological mechanism.

The strong neural-behavioral correlations ($r = 0.65-0.78$) exceed those typically reported in pediatric neuroimaging studies, possibly reflecting the specific matching of the intervention to the neural system being measured [19].

Study Limitations

Several limitations should be acknowledged. First, the relatively small sample size limits generalizability, though it was adequately powered for the primary hypothesis. Second, the 4-week intervention period was chosen based on adult studies but may not represent optimal dosing for children. Third, we did not assess long-term retention of gains, which is critical for establishing clinical utility.

The restriction to children with MACS levels I-III may limit applicability to more severely affected children. Additionally, the requirement for MRI compatibility excluded some potential participants, potentially biasing toward higher-functioning children.

Future studies should examine dose-response relationships, long-term outcomes, and applicability to children with more severe motor impairments. Integration with other neuroimaging techniques (e.g., diffusion tensor imaging, transcranial magnetic stimulation) could provide additional insights into plasticity mechanisms.

Research Implications

This study establishes a foundation for mechanism-based rehabilitation research in pediatric neurorehabilitation. The demonstration of target engagement (MNS activation) linked to functional outcomes provides a framework for

optimizing AOT protocols and developing biomarkers of treatment response.

Future research directions include:

Dose-response studies to optimize intervention parameters
Investigation of individual difference factors (e.g., lesion characteristics, genetic factors) that may influence AOT response.

Development of home-based AOT delivery systems using tablet technology.

Combination of AOT with other evidence-based interventions (e.g., constraint-induced movement therapy, bimanual training).

CONCLUSION

This randomized controlled trial provides the first direct evidence that goal-directed AOT produces functional improvements in children with UCP through activation of the MNS. The strong correlations between neural activation changes and functional gains validate the theoretical foundation of AOT and support its implementation in clinical practice. These findings establish AOT as a neuroplasticity-based intervention with clear mechanistic underpinnings, moving the field beyond purely behavioral outcome measures to include neurophysiological target engagement. The results support the incorporation of goal-directed action observation into rehabilitation protocols for children with UCP and provide a foundation for further optimization of this promising intervention approach.

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