INTRODUCTION

Cerebral Palsy (CP) is a multifaceted clinical condition that follows to fetal, perinatal, and even postnatal brain damage and is the most common form of chronic, motor, and postural disorder in the early years of life (Rosenbaum et al., 2007). The overall prevalence of CP is 2–2.5 per 1,000 live births and the risk increases up to 40–100 per 1,000 live births among premature or low birth weight infants (Johnson, 2002). Motor impairments can be either unilateral (i.e., hemiplegia) or bilateral, which are, respectively, related to a focal contralateral or bilateral brain injuries (Cans, De-la-Cruz, & Mermet, 2008). Although children with CP may achieve independent walking, many of them continue to be functionally impaired in daily-living activities involving more complex and precise bimanual tasks including reaching and grasping (Boxum et al., 2017).

To date, several motor interventions have been developed with the aim to improve upper limb motor functions, such as Constraint-Induced Movement Therapy (CIMT; Eliasson et al., 2018) and Hand-Arm Bimanual Training (HABIT; Bleyenheuft, Brandao, Bleyenheuft, & Gordon, 2015). CIMT...
aims to encourage spontaneous use of the affected upper limb by constraining the less-affected one (Hoare, Wasiak, Imms, & Carey, 2007), while HABIT focuses on improving the coordination of the two hands using intensive bimanual play and functional activities (Gordon, Schneider, Chinnan, & Charles, 2007). However, both motor interventions were developed for patients with unilateral CP, thus limiting their scope of use. Additionally, patients’ difficulties to tolerate the intensity and duration of these therapies, together with recent concerns about their actual efficacy are considered limiting factors to the adoption of these interventions in clinical practice (Manzoor, Kashif, Haroon, Dastgir & Iram, 2019; Sakzewski, Ziviani, & Boyd, 2014).

Recent discoveries in the neuroscientific field of motor cognition favored the development of a novel approach for the treatment of upper-limb motor impairments, known as Action Observation Treatment (AOT; Franceschini et al., 2010). The AOT capitalizes on the effects of action observation on mirroring mechanisms, which are already active early in development (Hunnius & Bekkering, 2014; Marshall & Meltzoff, 2014; Natale et al., 2014; Quadrelli & Turati, 2016). The neural signature of such mirroring mechanisms was provided by the seminal discovery of mirror neurons in the macaque premotor cortex (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992). Mirror neurons have the peculiar feature of firing both when the monkey performs a goal-directed action (e.g., grasping) and when the same or a similar action is perceived as performed by another agent. Importantly, a similar action-observation mechanism exists in the human brain: observing another individual performing an action activates in the observer the same motor cortical network recruited by motor execution (e.g., Keysers & Gazzola, 2010; Molenberghs, Cunnington, & Mattingley, 2012). Of relevance from a rehabilitation perspective, the mirror neuron system (MNS) is heavily involved in the human capacity to learn by imitation (Mattar & Gribble, 2005). In a similar vein, the inner motor simulation mechanism triggered by action observation would make the MNS suitable to serve as an alternative way to access an injured motor system, in turn reinforcing voluntary motor function (Buccino, Solodkin, & Small, 2006).

Based on these premises the AOT consists in the systematic observation of upper limb transitive actions (i.e., actions involving interactions with objects), selected based on their ecological value, and the subsequent reenactment of the same observed actions (Buccino et al., 2002; Ramachandran & Altschuler, 2009). The AOT was shown to be effective for motor rehabilitation in several clinical populations (Buccino, 2014), such as adult patients suffering from chronic stroke (e.g., Celnik, Webster, Glasser, & Cohen, 2008; Ertelet et al., 2007; Small, Buccino, & Solodkin, 2012) and Parkinson’s disease (e.g., Buccino et al., 2011; Pelosin et al., 2010), as well as in non-neurological populations undergoing the orthopedic surgery (Bellelli, Buccino, Bernardini, Padovani, & Trabucchi, 2010). In stroke patients, functional magnetic resonance (fMRI) studies proved the role of AOT in reorganizing sensorimotor areas, such as ventral premotor and supplementary motor cortex and the superior temporal gyrus (Ertelet et al., 2007).

A few experimental studies showed promising results with respect to the efficacy of AOT in improving motor planning and execution also in children with CP (Buccino et al., 2012, 2018; Kirkpatrick, Pearse, James, & Basu, 2016; Sgandurra et al., 2013). In Buccino and colleagues’ pilot study (2012), children with unilateral and bilateral CP aged 5–11 years were instructed to observe videos of daily uni- and bimanual actions and repeatedly reproduce those actions. Results showed an improvement of upper limb motor functions, as assessed with the Melbourne Assessment of Unilateral Upper Limb Function Scale (MUUL; Bourke-Taylor, 2003), as compared to controls. Using a similar protocol, Sgandurra and colleagues (2013) applied a 3-week AOT treatment to children with unilateral CP, aged 6–14. Following the treatment, participants showed a greater improvement in the spontaneous use of the impaired limb in daily activities, as assessed through the Assisting Hand Assessment (AHA); the clinical improvement was still present at 6-month follow-up. More recently, Kirkpatrick and colleagues (2016), assessed the effectiveness of a 3-months-long parent-delivered version of the AOT (i.e., repeated action observation followed by action execution), as compared to action execution alone, in children with unilateral CP aged 3–10 years. Results suggested that the home-based AOT lead to improvements in upper limb function that were analogous to those obtained after action execution alone. Thus, they did not confirm the AOT effectiveness as a supplementary intervention when delivered by parents at home. Overall, these results highlight the potential of AOT for driving the motor recovery in children with CP. However, the neural underpinnings of the AOT still remain to be determined. To date, only one fMRI study has explored the cortical reorganization following the AOT, showing an increased activation of premotor and parietal areas; both areas are involved in motor representation and are included in the human mirror system (Buccino et al., 2018).

Electroencephalography (EEG) is increasingly used in developmental and clinical populations to explore the neural networks involved in specific tasks. Unlike imaging techniques based upon hemodynamic responses, such as fMRI, EEG can give information about brain activity with higher time resolution (i.e., 1–2 ms), making it particularly valuable when assessing fluctuations in cortical dynamics involved in sensory and motor processes. Furthermore, because of its ease of use and the non-invasiveness, extensive research has focused on the identification of cost-effective EEG-based biomarkers that could be used for predicting the individual response to treatment in a wide variety of clinical populations.
formed before and after AOT. Fore and after each treatment, while EEG recording was performed in children with CP underwent 6 weeks of AOT, prior to improvement. To this aim, in a crossover design, a group of eight children was assigned to the control treatment (VOT) or the treatment group (treatment A) (Figure 1). For the allocation of the participants, a computer-generated list of random numbers was used. A therapist who was blind to the clinical aspects of the study, was involved in the functional assessments or the treatment procedures, performed randomization. Using a crossover design, both AOT as well as the VOT were performed subsequently in all children, following an AB/BA order. In particular, children who were randomly allocated to the AB arm received AOT during the 1st phase of the procedure, followed by VOT during the 2nd phase (AOT-VOT order). The treatments’ order was reversed in children assigned to the BA arm (VOT-AOT order). A washout period of 3 days was built into the trial at the end of the first phase of the procedure. Parents and children were blinded to group allocation (AOT-VOT or VOT-AOT) (Figure 2).

During the AOT, participants were involved in 18-min long, 3-days a week rehabilitation sessions for a duration of 6 weeks. Fifteen series of exercises, each consisting of three 20 s-long sequences, were video-recorded (Table 2). The
<table>
<thead>
<tr>
<th>ID</th>
<th>Treatment Order</th>
<th>Sex</th>
<th>CP type Hagberg</th>
<th>GMFCS</th>
<th>Motor abnormalities</th>
<th>GA (weeks)</th>
<th>BW (g)</th>
<th>Age (years)</th>
<th>MACS</th>
<th>IQ</th>
<th>Radiological findings (MRI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOT-VOT</td>
<td>M</td>
<td>T</td>
<td>4</td>
<td>Bilateral spasticity with truncal hypotonia, right side more affected</td>
<td>34</td>
<td>1,360</td>
<td>8</td>
<td>3</td>
<td>96</td>
<td>Bilateral periventricular leukomalacia at the peritrigonal area, bilateral parietal subcortical white matter hypotrophy, and thinning of the corpus callosum</td>
</tr>
<tr>
<td>2</td>
<td>VOT-AOT</td>
<td>F</td>
<td>SD</td>
<td>2</td>
<td>Bilateral spasticity, lower limbs more affected</td>
<td>27</td>
<td>760</td>
<td>6</td>
<td>2</td>
<td>82</td>
<td>Bilateral supratentorial periventricular white matter alteration, dilatation of lateral ventricles, white matter alteration in the centrum semiovale, liquoral space enlargement of the interhemispheric space, and thinning of the corpus callosum</td>
</tr>
<tr>
<td>3</td>
<td>AOT-VOT</td>
<td>F</td>
<td>SD</td>
<td>1</td>
<td>Bilateral spasticity, lower limbs more affected</td>
<td>31</td>
<td>1,290</td>
<td>6</td>
<td>2</td>
<td>74</td>
<td>Periventricular and posterior peritrigonal white matter signal hyperintensity</td>
</tr>
<tr>
<td>4</td>
<td>AOT-VOT</td>
<td>M</td>
<td>SD</td>
<td>1</td>
<td>Bilateral spasticity, lower limbs more affected</td>
<td>39</td>
<td>3,700</td>
<td>4</td>
<td>2</td>
<td>90</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>VOT-AOT</td>
<td>M</td>
<td>RH</td>
<td>1</td>
<td>Unilateral spasticity, right side more affected</td>
<td>34</td>
<td>1,750</td>
<td>4</td>
<td>2</td>
<td>90</td>
<td>Left periventricular gliosis associated with ventricular dilatation</td>
</tr>
<tr>
<td>6</td>
<td>VOT-AOT</td>
<td>M</td>
<td>LH</td>
<td>1</td>
<td>Unilateral spasticity, left side more affected</td>
<td>35</td>
<td>2,540</td>
<td>4</td>
<td>3</td>
<td>83</td>
<td>Right white matter periventricular gliosis with asymmetric ventricular dilatation, omolateral parietal white matter hypotrophy and thinning of the corpus callosum</td>
</tr>
<tr>
<td>7</td>
<td>AOT-VOT</td>
<td>M</td>
<td>SD</td>
<td>2</td>
<td>Bilateral spasticity, lower limbs more affected</td>
<td>27</td>
<td>675</td>
<td>13</td>
<td>3</td>
<td>74</td>
<td>Posterior periventricular white matter reduction and associated posterior ventricular dilatation</td>
</tr>
<tr>
<td>8</td>
<td>VOT-AOT</td>
<td>M</td>
<td>LH</td>
<td>1</td>
<td>Unilateral spasticity, left side more affected</td>
<td>38</td>
<td>3,400</td>
<td>13</td>
<td>3</td>
<td>117</td>
<td>Basal ganglia cysts and gliosis, retrograde axonal degeneration in correspondence of the right pons and mesencephalic structures, and thinning of the corpus callosum</td>
</tr>
</tbody>
</table>

Abbreviations: BW, birth weight; F, female; GA, gestational age; GMFCS, gross motor function classification system; IQ, intelligence quotient; LH, left hemiplegia; M, male; MACS, manual ability classification system; MRI, magnetic resonance imaging; NA, not available; RH, right hemiplegia; SD, spastic diplegia; T, tetraplegia.
viewed action could be both uni- and bi-manual, but were always goal-directed. Videos were always presented in the first-person perspective and they were customized in order to match and represent the impaired limb in case of unimanual exercises. The complexity of upper-limb movements displayed in the videos increased throughout the rehabilitative sessions (e.g., grasping and moving an object in the horizontal plane, or opening and closing a jar). After the observation of each video sequence for 1 min, children were asked to reproduce the observed action with their more-affected upper
limb, or with both limbs for bimanual actions, by the same objects employed in the videos for 2 min. Participants were assisted by a physiotherapist in order to keep their level of attention high and to control that they did not move their upper limbs, while watching the videos.

The control VOT was identical to the AOT with the exception that participants watched videos without any motor content representing car scenes extracted from videogames. Videos employed in the VOT were selected for their lack of motor connotation in order to avoid any potential uncontrolled training effect (i.e., activation of sensorimotor areas in response to human action observation). After observing the videos, children were asked to perform the same actions that were used during the AOT. As in the AOT condition, upper limb actions were performed upon receiving specific verbal instructions from the assisting physiotherapist (Figure 3).

### TABLE 2

| 1 | Grasping, opening a paper card and tilting the hidden colored card |
| 2 | Opening a box, grasping a toy and placing it on the table |
| 3 | Opening a stick of glue and gluing two cards together |
| 4 | Grasping some adhesive tape and sticking it on a vertical surface |
| 5 | Pouring some tempera from a bottle and dipping the thumb to leave the fingerprint on a paper sheet |
| 6 | Pouring some tempera from a bottle and dipping the index to leave the fingerprint on a paper sheet |
| 7 | Pressing the soap dispenser |
| 8 | Opening the tap water and rubbing your soaped hands under water |
| 9 | Taking a piece of absorbent paper from the roll, tearing off the piece of paper and rubbing your hands to dry |
| 10 | Grasping a writing case, opening the zipper to grab a sharpie |
| 11 | Drawing a circle after opening a sharpie |
| 12 | Grasping a paper card, folding and duck-taping it to make a crown |
| 13 | Grasping a wire to insert it into a hole |
| 14 | Grasping a button and thread a wire through it |
| 15 | Grasping a paper crown to place it on a second person’s head |

## 2.3 Upper limb function assessments

The current sample comprised children with both bilateral and unilateral CP, thus requiring functional evaluations to be completed during bimanual performance using the Both Hands Assessment (BoHa; Elvrum, Zethraeus, Vik, & Krumlinde-Sundholm, 2018). However, as no validation of the BoHa was available at the time of data collection, the functional assessment was carried out using the AHA (Krumlinde-Sundholm, Holmefur, Kottorp, & Eliasson, 2007) and the MUUL (Bourke-Taylor, 2003). The AHA provides a measure of how effectively children with CP can use their affected hand during bimanual tasks, while the MUUL is a test comprising 16 items quantifying the quality of upper limb motor functions. The AHA and MUUL were administered 6 weeks and 1 day before the onset of the treatment (T0 and T1, respectively; i.e., baselines), at the end of the first 6 weeks of treatment (T2), and 1 day and 3 months after the end of the entire experimental procedure (T3 and T4, respectively).

Therapists assisting during the intervention were not blind to group allocation; instead, therapists performing the
2.4 EEG recordings and analysis

Electrophysiological measurements were performed before and at the end of AOT (i.e., T1 and T2 for the AOT-VOT group; T2 and T3 for the VOT-AOT group). During the EEG recording, children sat in a dimly lit room at a viewing distance of 90 cm from a 20” CRT monitor. Stimulus presentation was controlled by E-Prime 2.0 software (Psychology Software Tools, Inc., Sharpsburg, PA), which sent event timing information to the EEG acquisition computer at the onset of each stimulus. The experimenter monitored the participant’s looking behavior using a video-camera. The stimuli were 3-s video-clips of a hand performing a reach-to-grasp action with a precision grip. The videos were edited to show only the experimenter’s left or right arm and hand in a first-person perspective against a dark background. The choice of using the right or left limb for each participant was customized so that each participant was presented with videos of the limb that was more impaired according to the functional evaluations. The reach-to-grasp actions were directed toward different objects (i.e., a small cube or a mug being grasped by its handle). Videos were presented in a random order, according to the grasped object, and were always preceded by an intertrial interval, consisting of a fixation cross, lasting randomly between 1,200 and 1,600 ms. Participants were instructed to sit and remain as still and attentive as possible, while watching each video for its entire duration.

EEG was recorded using a Brain Quick Micromed 98 System with 19 electrodes placed according to the 10–20 international system, sampled at 256 Hz and stored on the hard disk for further analysis. The signal was recorded with respect to the FpZ electrode and re-referenced to the average
of all electrodes. A bandpass filter of 0.8 to 70 Hz, together with a 50 Hz notch filter, was applied online and impedances were checked prior to the beginning of each session and considered acceptable if lower than 20 kΩ. EEG data were segmented into 3,400 ms trials beginning 1,000 ms before and ending 2,400 ms after the stimulus onset.

The mean number of presented trials was 49.4 (SD = 10.6) at the pretreatment session and 48.6 (SD = 6.3) at the posttreatment session. However, to ensure that we were measuring sensorimotor activation in response to the observation of movement, rather than as a consequence of participants’ own concurrent movements, a careful procedure for eliminating movement artifacts was adopted. This procedure consisted in the video-recordings of the sessions, through a camera hidden over the monitor. Offline analyses of the video allowed us to exclude trials in which children made any gross or fine movements or trials in which the children were not attending to the screen. After such artifact rejection procedure, the mean number of artifact-free trials contributing to analyses was 23 (SD = 7.4) at the pretreatment session and 21.9 (SD = 8) at the posttreatment session. Time-frequency analyses were performed on each artifact-free trial using continuous wavelet transform with Morlet wavelets at 1 Hz intervals in the 3 to 20 Hz range. Following the procedure used in previous studies investigating µ rhythm suppression (e.g., de Klerk, Johnson, & Southgate, 2015; Pomiechowska & Csibra, 2017; Quadrelli, Geangu, & Turati, 2019; Quadrelli, Roberti, Turati, & Craighero, 2019) or performing time-frequency analysis to uncover other stimulus-induced oscillatory responses (e.g., Csibra, Davis, Spratling, & Johnson, 2000; Parise & Csibra, 2013), we calculated the absolute value (i.e., the amplitude, not the power) of the resulting complex coefficients. In order to eliminate distortion created by the wavelet transform, the first and the last 400 ms of each trial were removed and a 500 ms baseline period starting 600 ms before the stimulus onset was selected. Averaged activity in the 8–13 Hz range during the 500 ms baseline period was then subtracted from averaged activity recorded during stimulus presentation. Average wavelet coefficients within children were calculated by taking the mean across the trials. Based on previous work showing that in children of this age the most reactive frequency band to movement observation is 8–13 Hz (Oberman et al., 2005), we averaged activity over this range.

As in existing studies investigating sensorimotor alpha suppression (Lepage & Theoret, 2006) in childhood, activity from C3 and C4 electrodes, disposed over the left and right arm/hand representation areas was analyzed and compared to activity recorded from Cz, disposed over the foot representation area. We compared sensorimotor alpha suppression at electrode sites overlying the hand areas (C3 and C4) with activation recorded over the foot area (Cz) of the sensorimotor cortex in order to verify whether the efficacy of AOT would be specific to hand areas or would result in a broader recruitment of sensorimotor areas. The average activity in the alpha range (8–13 Hz) was extracted for statistical analyses from these regions in a 500–1,500 ms time window. This time window includes the reaching part of the movement as well as the preshaping of the hand into a pincer grip to grasp the object. EEG data were preprocessed using EEGLAB v13.5.4 (Delorme & Makeig, 2004) and analyzed using WTools (see Parise & Csibra, 2013).

Seven out of 8 enrolled children also provided enough trials to perform EEG analysis on the reorganization of sensorimotor activity following AOT. One participant was excluded from analysis performed on the EEG data due to the lack of attention toward the videos and presence of excessive movements during the task.

2.5 Preliminary analysis

In a preliminary set of analyses we examined the presence of potential differences between functional scores at the AHA and MUUL by means of Wilcoxon matched-pairs signed-rank tests between the two baseline evaluations (T0 and T1). No significant differences were found between the two baseline scores at the AHA, $Z = 10, p = .10$ (T0: $M = 64.60, SD = 9.69$; T1: $M = 63.90, SD = 9.54$) and at the MUUL for both the more-impaired limb, $Z = 20.5, p = .78$ (T0: $M = 73.30, SD = 15.72$; T1: $M = 72.4, SD = 15.85$) and the less-impaired limb, $Z = 1.0, p = .22$ (T0: $M = 95.60, SD = 5.84$; T1: $M = 97.10, SD = 4.43$). Moreover, we also compared functional scores at the AHA and MUUL at T0 and T1 between the two experimental groups (i.e., AOT-VOT vs. VOT-AOT) by means of Mann-Whitney $U$ tests in order to explore eventual unbalances in distribution of participants among the two groups. No significant differences were highlighted between the two groups at the AHA at T0, $U = 4.50, p = .38$ (AOT-VOT: $M = 68.0, SD = 6.48$; VOT-AOT: $M = 61.3, SD = 12.12$) and T1, $U = 4.50, p = .38$ (AOT-VOT: $M = 67.30, SD = 6.34$; VOT-AOT: $M = 60.50, SD = 11.90$). No significant differences emerged at the MUUL for the more-impaired limb at T0, $U = 8.0, p = .10$ (AOT-VOT: $M = 77.70, SD = 12.88$; VOT-AOT: $M = 68.80, SD = 18.90$) and T1, $U = 5.00, p = .49$ (AOT-VOT: $M = 76.10, SD = 13.85$; VOT-AOT: $M = 68.70, SD = 18.90$). No significant differences were found at the MUUL also for the less-impaired limb at T0, $U = 6.00, p = .62$ (AOT-VOT: $M = 94.70, SD = 7.74$; VOT-AOT: $M = 98.40, SD = 3.28$) and at T1, $U = 5.00, p = .41$ (AOT-VOT: $M = 95.30, SD = 5.70$; VOT-AOT: $M = 99.00, SD = 2.05$) (see data in Supplemental Table 1).

Following this preliminary set of analyses, we aimed to assess whether AOT improves hand function in our sample. Given that no significant differences were highlighted between the two baselines and between the two groups, we matched the MUUL and AHA scores at each time point for
each participant in the two groups. Data for the matched functional scores were further analyzed by means of Wilcoxon matched-pairs signed-rank tests as a function of AOT or VOT (i.e., Pre-1day vs. Post-2days as a function of AOT or Pre-1day vs. Post-2days as a function of VOT). Furthermore, the presence of functional differences at the MUUL and AHA between the two treatments was explored by means of Wilcoxon matched-pairs signed-rank tests comparing functional scores before and after AOT to scores obtained, respectively, before and after VOT.

After demonstrating the clinical effects of AOT, EEG data were analyzed to compare the scalp distribution of sensorimotor alpha suppression over central electrode sites in response to the observation of precision grip hand actions before and after the AOT. To this aim, we used Wilcoxon matched-pairs signed-rank tests to compare activation observed before (i.e., Pre-AOT) and at the end of AOT (i.e., Post-AOT) separately over C3/C4 and Cz. Lastly, we sought to explore the correlation between sensorimotor activity and functional improvements. Difference scores for both electrophysiological (i.e., sensorimotor activity) and functional data (i.e., MUUL and AHA) were calculated as: \( \Delta = \text{score at Post-AOT} - \text{score at Pre-AOT} \).

Statistical analyses were performed using SPSS 25 (IBM Corp., Armonk, NY, USA) and conducted on a .05 level of significance (2-tailed). Pairwise comparisons were performed and corrected using the Holm–Bonferroni adjustment procedure. Furthermore, in order to strengthen our results, the classic frequentist analyses were complemented with the same analyses performed under a Bayesian approach in Jamovi 1.0 (https://jamovi.org) by using the default Cauchy prior \( r = .707 \). Using the Jamovi formalism, the index next to the Bayes Factors (BF) indicates that the null hypothesis \( (H_0) \) is in the denominator and the alternative hypothesis \( (H_1) \) is in the nominator. Thus, \( BF_{10} = \frac{p(\text{data}|H_1)}{p(\text{data}|H_0)} \), with \( BF_{10} > 10 \) that is considered strong evidence for an effect, \( 3 < BF_{10} < 10 \) that is considered moderate evidence for an effect.

3 | RESULTS

3.1 | Assisting Hand Assessment

Analyses on the AHA scores showed a significant difference between the pre- and post-AOT evaluations, \( Z = 9.00, p = .01, d = 3.18 \) (pre-AOT: \( M = 63.90, SD = 9.08 \); post-AOT: \( M = 76.30, SD = 7.03 \)), while no differences were highlighted between the pre- and post-VOT evaluations, \( Z = 2.50, p = .06, d = .37 \) (pre-VOT: \( M = 69.30, SD = 12.93 \); post-VOT: \( M = 68.30, SD = 12.00 \)). Notably, the comparison between the post-AOT and post-VOT evaluations was significant, \( Z = 21.00, p < .05, d = 1.07 \) (post-VOT: \( M = 68.30, SD = 12.00 \); post-AOT: \( M = 76.30, SD = 7.03 \)). All other comparisons did not attain statistical significance (all \( ps > .08 \)). Two-tailed paired sample Bayesian \( t \)-tests confirmed the frequentist results showing very strong evidence for a difference in the AHA scores from pre- to post-AOT evaluations (\( BF_{10} = 557.29 \)) and moderate evidence for a difference between post-AOT and post-VOT scores (\( BF_{10} = 3.92 \)). Overall, results show that significant improvements at the AHA emerge only after the AOT (Figure 2).

3.2 | Melbourne assessment of Unilateral Upper Limb function

Analyses performed on the MUUL scores for the more-affected limb revealed a significant difference between the pre- and post-AOT evaluations, \( Z = 4.72, p < .01, d = 1.67 \) (pre-AOT: \( M = 73.20, SD = 15.51 \); post-AOT: \( M = 87.20, SD = 8.65 \)), while no difference was highlighted between the pre- and post-VOT evaluations, \( Z = 16.50, p = .74, d = .21 \) (pre-VOT: \( M = 79.20, SD = 17.38 \); post-VOT: \( M = 78.20, SD = 16.30 \)). Notably, the comparison between the post-AOT and post-VOT evaluations was significant, \( Z = 2.64, p < .05, d = .94 \) (post-VOT: \( M = 78.30, SD = 16.30 \); post-AOT: \( M = 87.20, SD = 8.65 \)). All other comparisons did not attain statistical significance (all \( ps > .21 \)). Two-tailed paired sample Bayesian \( t \)-tests confirmed the frequentist results showing strong evidence for a difference in the MUUL scores from pre- to post-AOT evaluations (\( BF_{10} = 22.11 \)) and anecdotal evidence for a difference between post-AOT and post-VOT scores (\( BF_{10} = 2.58 \)) (Figure 3).

Frequentist analyses performed on the MUUL scores for the less-affected limb did not attain statistical significance (all \( ps > .18 \)) and were further confirmed by low \( BF_{10} \) values (all \( BF_{10} < 1.08 \)).

Overall, the analysis of the clinical data (AHA and MUUL) speak in favor of the clinical effectiveness of the AOT, as compared to the control VOT: we found significant improvements at the AHA and the MUUL, which arise only after the AOT, but not after the VOT, and which pertain the more-affected limb only. Moreover, all patients improved more than the recommended smallest detectable change following to the AOT for the AHA scores (i.e., >5 units; Krumlinde-Sundholm et al., 2007), and 6 out of 8 patients also showed a clinically relevant improvement at the MUUL for the more-impaired limb (i.e., 8.9%; Klingels et al., 2008).

3.3 | EEG Results

Preliminary analyses revealed no significant differences in sensorimotor activation between C3 and C4 and no differential patterns of results between these two electrode clusters.
(all ps > .11). Thus, similarly to previous studies (e.g., Saby et al., 2013), sensorimotor alpha suppression from C3 and C4 were averaged to index sensorimotor activation over the hand areas (see Supplemental Figure 1).

Paired-sample t-tests comparing activation recorded over C3/C4 and Cz revealed that sensorimotor activation recorded over C3/C4 electrodes was significantly greater at post-AOT ($M = -0.31 \mu V; SD = 0.19 \mu V$) compared to pre-AOT ($M = -0.06 \mu V; SD = 0.14 \mu V; Z = 28.00, p = .01, d = 1.13$), while no difference was found over Cz between pre-AOT ($M = -0.18 \mu V; SD = 0.24 \mu V$) and post-AOT activation ($M = -0.27 \mu V; SD = 0.23 \mu V; Z = 19.00, p = .49, d = .31$) (Figure 4). Two-tailed paired sample Bayesian t-tests confirmed the frequentist results showing moderate evidence for a difference in the sensorimotor activation over C3/C4 from pre- to post-AOT ($BF_{10} = 3.40$) and no evidence for a difference over Cz between pre- and post-AOT ($BF_{10} = 0.46$).

Additionally, one sample t-tests were performed to investigate the magnitude of sensorimotor activation as compared to baseline in both electrode clusters and at both pre- and posttreatment. Sensorimotor activation was significantly different from zero over C3/C4 ($M = -0.31 \mu V; SD = 0.19 \mu V; Z = -4.40; p = .02, d = 1.65$) and it was marginally different over Cz ($M = -0.28 \mu V; SD = 0.23 \mu V; Z = 2.00; p = .05, d = 1.18$) at posttreatment. No significant activation was found at pretreatment over both electrode clusters (all ps > .09; all ds < .77). Two-tailed one sample Bayesian t-tests also confirmed the frequentist results showing strong evidence for a difference in the sensorimotor activation over C3/C4 compared to baseline ($BF_{10} = 11.94$) and moderate evidence for a difference over Cz compared to baseline ($BF_{10} = 3.90$).

Lastly, a significant correlation between MUUL gain scores for the more-impaired limb and sensorimotor differential activation was found over C3/C4, $r = .79; p = .03, z = 1.12$ (all other ps > .16). This correlation was further supported by the corresponding Bayesian analysis showing moderate evidence for correlation ($BF_{10} = 3.00$). In other words, greater differential sensorimotor activation over C3/C4 was associated with greater functional improvement as assessed by the MUUL for the more-impaired limb (Figure 5).

### 4 | DISCUSSION

The present research investigated whether the clinical beneficial effects following AOT may be mediated by the modulation of sensorimotor areas activation, as indexed by
Changes in $\mu$ rhythm oscillations during a passive action observation task. To this aim, we assessed the relationship between sensorimotor activation and clinical improvements of upper limb motor functions in 4- to 14-year-old children with CP.

Results suggest for the first time that AOT can improve upper limb functions in CP by modulating the mirror activation of the sensorimotor network. Indeed, following AOT, $\mu$ rhythm suppression is selectively increased in electrode locations located over the hand areas of the sensorimotor cortex. Since $\mu$ rhythm suppression is related to the activation of the mirror system during observation and performance of goal-directed actions, the AOT-induced increase in $\mu$ rhythm suppression over C3/C4 can be ascribed to the improved activation of motor areas. This result is in line with findings from fMRI studies in both children with CP (Buccino et al., 2018) and adult stroke patients (Ertelt et al., 2007) showing the increased activation of frontal and parietal areas (in particular of the premotor cortex and the superior temporal gyrus) following AOT. Interestingly, despite no significant difference was found between sensorimotor activation recorded over Cz between pre- and post-AOT, we reported a significant absolute activation (i.e., compared to baseline) over Cz at post-treatment. One possible interpretation for this finding is that the observed activation was due to the modulation of a wider portion of the sensorimotor cortex after treatment. Indeed, it was previously demonstrated that children with CP show a more diffuse $\mu$ band perturbation compared to healthy children during the execution of reach-to-grasp movements (Lee et al., 2012). In this vein, the significant post-AOT activity observed over C3-C4 and Cz can be ascribed to cortical activation deriving from more diversified regions being tackled by the AOT (e.g., sensorimotor cortex, supplementary motor area, and posterior parietal cortex). Additionally, the relationship between motor improvement assessed with the MUUL in our sample and the concurrent increase of $\mu$ rhythm suppression over C3/C4 indicates that the greater the sensorimotor activation brought about AOT, the greater the improvement in the quality of more-impaired upper limb functionality as assessed with the MUUL. Thus, our study highlights the link between clinical efficacy of AOT and the underlying reorganization of sensorimotor mirroring mechanisms: action observation may serve as an alternative mean to access an injured motor cortical system to rebuild voluntary motor control in CP (Burzi, Tealdi, Boyd, & Guzzetta, 2016).

Furthermore, results from the present study, although coming from a small clinical sample, confirm the therapeutic potential of AOT for the treatment of upper limb motor performance. Specifically, children with CP showed improvements at both MUUL and AHA scores only after AOT, as compared to pre-AOT and to post-VOT evaluations, in line with previous evidence (Buccino et al., 2012; Sgandurra et al., 2013). Importantly, functional improvements following AOT cannot be ascribed to pre-existing spontaneous fluctuations within our sample. Indeed, it was established that there was no improvement (nor decline) in AHA and MUUL scores between the two pretreatment baselines. In particular, the results relative to the AHA indicate that children with CP can improve the efficiency of their affected hand in bimanual activities following AOT. Moreover, the enhanced spontaneous use of the affected limb is specific for AOT, given that no differences in AHA scores were found after the control treatment (i.e., VOT). Interestingly enough, the observed improvement following AOT was greater than the recommended smallest detectable change for all patients. The same pattern emerges for MUUL scores, where improvements of the quality of upper limb motor functions were induced only by AOT, but not by VOT. These results extend those obtained in previous research (Buccino et al., 2012, 2018; Sgandurra et al., 2013). Indeed, MUUL results showed specific functional gain following the intervention, and also met the requirements for an authentic clinical improvement.

It is to note that the functional improvement observed in our patients cannot be merely attributed to the daily execution of hand actions during the AOT, as the amount of functional improvement following the control, VOT, treatment was not comparable to that observed after AOT. While the same amount of motor exercise was required in the VOT and AOT, they only differed in terms of absence/presence of the action observation component. This further supports the view that it is the “mirror” component of the AOT that played a key role in driving the functional improvement observed in the current study. As a side clinical consideration, differently from other existing interventions, the main focus of AOT is not on action execution, but on action observation. This aspect might help patients with CP to overcome excessive
frustration deriving from not being able to perform an action. In turn, lowering frustration levels might enhance commitment and compliance from patients, rendering AOT easier to accept and tolerate.

Overall, results from the current study provide preliminary evidence in support of the potential value of EEG, and specifically \( \mu \) rhythm desynchronization, as an electrophysiological correlate of AOT outcome in children with CP. Furthermore, the present findings, although only based on a small sample, support the use of action observation as a strategy to enhance motor rehabilitation in children with CP and provide further evidence that AOT might be implemented to the standard concepts of physiotherapeutic practice. Future larger studies in children with CP will be important to further clarify the picture of the electrophysiological correlates of AOT-based clinical improvements in cerebral palsy. Future research should also help to discern the role of the observation and execution phases of AOT in the reorganization of sensorimotor areas. In the current study, no EEG sessions were performed at pre- and post-VOT. This implies that similar to Buccino and colleagues (2018), it is not possible to definitely disentangle whether sensorimotor modulation observed after AOT was due to the observation alone or to the combination of observation and execution. Moreover, it will also be important to better characterize the existence of individual differences in frequency, topography and modulation patterns in children with CP and to explore the effects of such differences on functional reorganization mechanisms following AOT or other motor rehabilitation paradigms (see Démas et al., 2019).

Our small sample size does not allow us to draw definitive inferences at the population level. Studies including a larger number of children with CP are necessary to corroborate the current preliminary findings. A potential weakness that is linked to the limited sample size and to the consequent decision to match functional scores at each time point for each participant in the two groups, is that it was not possible to verify the long-term effects of AOT on functional recovery of upper limbs. Existing studies provide mixed results in this sense. For example, Buccino and colleagues (2018) demonstrated that the positive effects of AOT were sustained up to the 2-months follow-up, while in another study employing a home-based, parent-delivered version of AOT, no significant differences were reported at the 3- and 6-months post-treatment evaluations between the control and experimental groups (Kirkpatrick et al., 2016). It is plausible that this difference in the long-term efficacy of AOT is due to dissimilarities in treatment delivery, duration and intensity; however, further studies are needed to assess the long-term effects of AOT on upper limb motor improvements. In addition, similar to Buccino and colleagues (2012), the heterogeneity of the CP sample in terms of the type of paralysis jointly with its sample size, prevented us to investigate the impact of specific characteristics related to CP on the functional and electrophysiological outcomes of AOT. However, it is to note that in all patients within our sample, including those with tetraplegia, upper-limb deficits appeared to be lateralized, with one limb being more impaired than the other. While the small sample size and the very short-term follow-up deriving from the need to increase statistical power in the analyses represent significant limitations of our pilot study, its strength includes the use of reliable functional measures together with the use of novel electrophysiological indices.

In conclusion, AOT appears to be a promising rehabilitation tool for children with CP, since it may drive the reorganization of sensorimotor areas, as indexed by the increased \( \mu \) rhythm desynchronization at electrodes overlying the upper limb areas, which were the target of the action observation training. Despite being preliminary, the present evidence supports the view that AOT can favor motor recovery in clinical populations by affecting cortical plasticity. Indeed, current EEG results seem to call into question the same cortical networks as in previous fMRI studies in stroke patients (Ertelt et al., 2007) and children with CP (Buccino et al., 2018), suggesting that AOT is capable to contribute to rebuild physiological sensorimotor circuits (e.g., Buccino et al., 2018; Ertelt et al., 2007). Thus, besides confirming its clinical potential, the present study also highlights that EEG could be useful to identify electrophysiological correlates of the plasticity occurring within the sensorimotor cortex following AOT in children with CP. Although current results do not have yet direct clinical relevance to the prediction of individual cortical reorganization in response to AOT, they lead the way for further research into the functional significance and potential clinical implementation of AOT. Future studies might examine the effects of shorter AOT sessions on the modulation of sensorimotor activity in children with CP and assess the long-term maintenance of the effects of AOT on cortical reorganization. Lastly, it might also be of interest to assess the role of individual differences, such as age or the specific form of CP, in modulating the effects of AOT on functional and electrophysiological outcomes.

ACKNOWLEDGMENTS
This work was supported by a European Research Council Starting Grant to Prof. Chiara Turati on a project entitled “The origins and development of the human mirror neuron system”—ODMR No. 241176.

CONFLICT OF INTEREST
The authors whose names are listed above certify that there are no affiliations with or involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript (e.g., employment, consultancies, stock ownership, honoraria, and/or expert testimony).
DATA AVAILABILITY STATEMENT
Data cannot be made publicly available as the dataset contains sensitive and identifying information. The authors confirm that the data and experimental stimuli will be made available upon request. Requests may be sent to the corresponding author.

ORCID
Ermanno Quadrelli https://orcid.org/0000-0002-6834-0912

REFERENCES


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